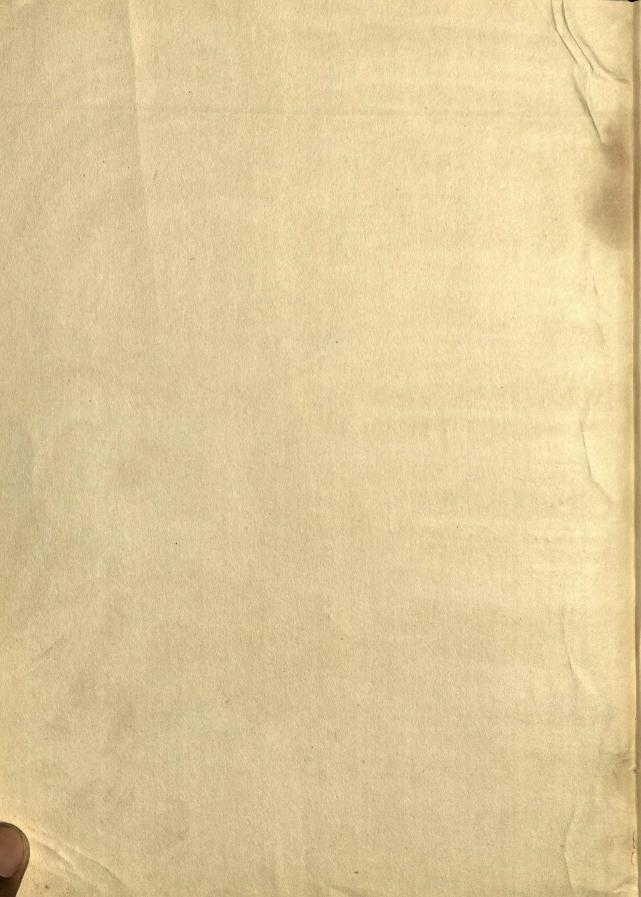
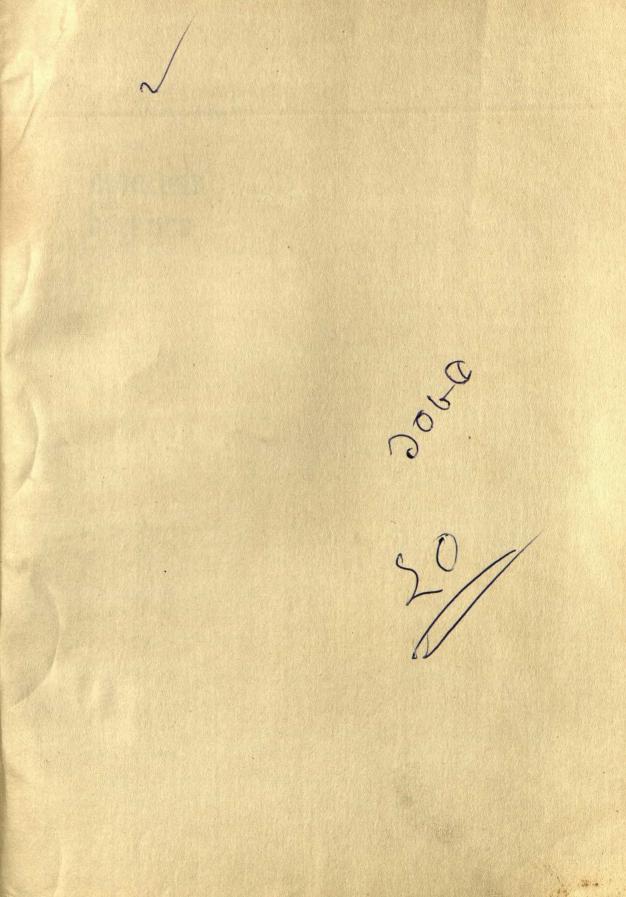
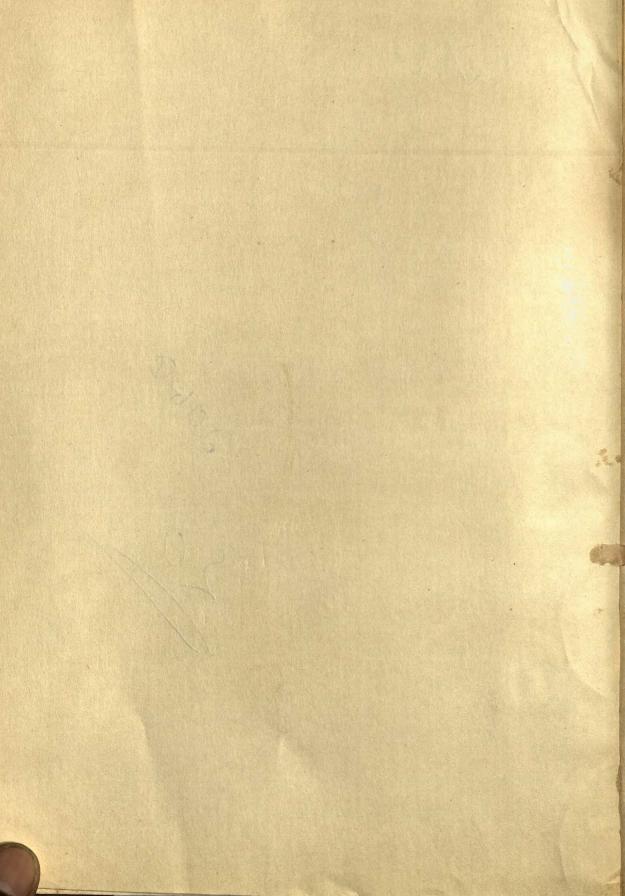


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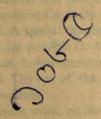
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V R DNYANSAGAR
Department of Botany
Nagpur University





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Published by Tata McGraw-Hill Publishing Company Limited 4/12 Asaf Ali Road, New Delhi-110 002, and printed at

Dominant Offset Printers B-36, Group Inds, Wazirpur, Pelhi-52

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ply wife a usual set daugater stuche neighbor inche various ways during the preparation While teaching cytology and genetics in B Sc classes, I felt the need for a suitable textbook which would include both these branches of biology. I had prepared detailed notes on these subjects, which proved very useful to my students. So, when I was assigned the task of writing a book on cytology and genetics in Marathi for BSc students, these notes became the basis of my work. At this time, I was also thinking of writing a similar book in English. It was only when Tata McGraw-Hill Publishing Co. Ltd, New Delhi, showed keen interest in publishing this book that I earnestly started on the work of writing it.

D. VS Kotlest, Merthwell University, Dr.A.D. Choudnaty, (Magnett University). Shir Prayeen Chandra and select Sciences (Inchante of Science, Name - helped me much in the preparation at the biliness row, and the Charachary in going theorem the representate the

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Both cytology and genetics have made tremendous progress in recent years. Some discoveries in the field of genetics have been so startling that even laymen have evinced keen interest in them. It is, therefore, obvious that B Sc students pursuing the study of cytology and genetics are expected to possess information in some detail. A special chapter on genetic engineering has been included in this book for them. It is often not possible for the majority of students to purchase books dealing with cytological techniques. It was therefore thought that including a chapter on cytological techniques would be useful. The rest of the chapters deal with the topics prescribed in the syllabi of most Indian Universities.

I have made every effort to include the latest relevant work, on the basis of available literature, so as to make this book as up to date as possible. In addition to the comprehensive bibliography given at the end, selected references (selective reading) are given at the end of each chapter. Teachers should encourage students to refer to such literature. A summary is given at the end of each chapter. It is hoped that this will prove useful to students while

revising each topic.

I take this opportunity to express my grateful thanks to the National Book Trust, New Delhi, for subsidising the publication.

I am grateful to Prof. G B Deodikar, Retd Director, Maharashtra Association for Cultivation of Science (MACS), Pune; Prof. S S Bir, Head of the Botany Department, Punjabi University, Patiala; and Dr R V Pandit, Veterinary College, Nagpur, for valuable suggestions.

I am obliged to Prof. G B Deodikar and Dr V P Patil of MACS, Pune; Dr Joginder Singh, Indian Agricultural Research Institute (IARI), New Delhi; Prof. R P Roy, Patna University, Patna; Dr SH Tulpule (Botany Department) and Dr AK Indurkar (Zoology Department), Poona University, Pune; Dr AB Sapre, Marathwada University, Aurangabad; Dr Kakde, Associate Dean and Prof. S B Lal, Punjabrao Agriculture University (College of Agriculture, Nagpur) and my old students, viz. Dr A Dhanaraj, Dr C D Chowta, Dr A R Pingle and Dr R J Thengane (Poona University) for photomicrographs and photographs. I am also thankful to Shri Vilas Andhare, Director, Maharashtra Universities Book Production Board, Nagpur; Dr K M Mann, Editor, Scope Publications (Upjohn Co., Kalamazoo, Michigan, USA); Prof. R A Popham, Business Manager, American Journal of Botany and McGraw-Hill

Book Co., for permission to reproduce certain figures.

Dr SH Tulpule, Dr V L Bakale (Institute of Science, Nagpur), Dr R J Thengane, Dr V S Kothekar (Marathwada University), Dr A D Choudhary, (Nagpur University), Shri Naveen Chandra and Shri T Srinivasu (Institute of Science, Nagpur) helped me much in the preparation of the bibliography, and Dr Choudhary in going through the typescript. I extend my grateful thanks to them.

My wife Kusum and my daughter Rucha helped me in various ways during the preparation

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## Introduction

#### Genetics

Genetics is defined as the science of heredity. It considers the transmission of characters from generation to generation. It was in 1906 that Bateson proposed the term 'genetics' for that branch of biology which deals with "the elucidation of the phenomena of heredity and variation." Although the history of genetics can be traced from the ancient period, its background was laid only in the seventeenth century, and modern genetics was born when Mendel's paper embodying the results of his hybridization experiments was published in 1866. Mendel is regarded as the Father of Modern Genetics as his paper laid the scientific foundation of genetics. However, his work remained unknown to the scientific world for 34 years. In 1900, his paper was rediscovered independently by De Vries, Correns and Tschermak. Genetics is, therefore, one of the younger branches of the biological sciences. However, its progress has been astonishingly rapid, especially after the Second World War.

#### Cytology

Cytology is the science of cells. Since the study of cell requires a microscope, the history of cytology began only after the invention of the microscope. It had its beginning in 1665, when Robert Hooke first observed cork cells under a microscope made by him. After this a series of studies followed leading to the formation of the cell theory by Schleiden in 1838. According to this theory, all organisms are made of cells. It was made applicable to animals by Schwann in 1839. The cell theory was regarded as one of the great generalisations of experimental biology. In 1858, Virchow established conclusively that a cell never arises de novo, but that it arises from another cell (the theory of cell ineage). In the beginning of the twentieth century, the research stream took a turn when a close link between embryology and genetics was discovered. In 1902, Sutton, who was the first to propound the chromosome theory, pointed out the parallelism of behaviour between chromosomes in meiosis and the Mendelian factors in heredity. Thus cytology was linked to genetics and the branch of cytogenetics was born.

#### History of Cytology

The period of the nineteenth century is considered as the classical period of cytology as it was during this period that its foundations were being laid. The modern period begins with the rediscovery of Mendel's work on genetics in 1900. Cytology therefore had a beginning with a very long descriptive period passing into the modern experimental period.

However, one must not forget that the cytologist is primarily a morphologist. As such, descriptive cytology continues to be an important part of cytological research. O. Hertwig and R. Hertwig's studies on fertilisation in 1887 marked the beginning of experimental cytology. When Wilson's The Cell in Heredity and Development was published in 1925, the relationship between cytology and genetics had become closer. The development of the chromosome theory by the Drosophila workers (1910-20) of the Morgan school, the discovery of giant chromosomes, the use of heteroploidy and polyploidy as cytogenetic tools, and the advances made in the cytogenetics of maize contributed to the creation of more avenues of approach towards a better understanding of the role of chromosomes in cell division and heredity.

The present phase of experimental cytology is actually an extension of the earlier work. During and after the Second World War, cytological researches have been chiefly on the chemical side and a branch called cytochemistry or biochemical cytology has developed. With the isolation of DNA (the hereditary substance) by Avery. Macleod and McCarty in 1944 and the model of its structure proposed by Watson and Crick in 1953, the researches have been mostly on the (i) nature of gene, (ii) its mode of replication, and (iii) its manner of action and the determination of characters.

Our knowledge of intercellular activities has now advanced in a spectacular manner. After the formulation of the concept of catalytic activity by Ostwald and the discovery of cell enzymes, physics and chemistry have been increasingly employed for researches on cell physiology, and cytology has now come to be known as cell biology.

As regards energy input, plants and animals resemble each other closely apart from the direct source of energy upon which they have to depend. The former are autotrophs and the latter heterotrophs. However, it should be noted that the living matter is capable of presenting its highly improbable-appearing individuality at the expense of large amounts of free energy obtained from the environment. It has been shown that the laws of thermodynamics can be applied to the living matter, meaning thereby that it is an integral part of the physical world. Studies are also concentrated on the mechanism by which enzymes and other proteins are manufactured in a living system and how information for their synthesis is carried from one generation to the other.

With finer cytological techniques, single cells as well as their parts can now be isolated. With the invention of the electron microscope, the study of cells in minute details started and it has led to tremendous progress in cell biology. It has now been possible to resolve cell structure at the molecular level, and a separate branch called molecular biology has come into existence. Although it may appear that the development of separate branches such as cytochemistry, cytogenetics, or molecular biology may result in narrow specialisations, they are actually tending to approach unity, since the cell is the common denominator or basic module of the living system.

Information on the nature and manner of transmission of genetic specificity (chromosomal heredity) prior to 1940 was based on the series of developments of the theory of the gene. Müller started studies on mutation in 1920, and in 1927 he discovered the mutagenic effects of radiation in *Drosophila*, and stadler in maize. This discovery was a significant step in the progress of genetics. Just prior to the Second World War, ideas from physics

were introduced by workers like Frank-Kammenetski, Friedrich-Freska, Jorden and Zimmer-Delbruck in the sphere of genetics, especially in connection with the problem of size, mutability and self-replication of genes. Müller and Timofeef-Ressovsky acted as biological interpreters. Morgan put forth an ingenious idea of crossing over and suggested that the genes are situated in the chromosome in a linear fashion. This formed the basis of the classical concept of the gene according to which the sene is the ultimate unit of heredity, mutation and recombination. A gradual change has taken place in the outlook on genetics, especially the concept of gene, since 1940. Though the classical theory of gene is convenient to use in everyday genetics, it is no longer tenable as the works of Benzer, Bonner, Pontecarvo have shown that the gene is further divisible. Benzer has suggested the terms muton, recon and cistron with reference to mutation, recombination and function, respectively.

The present astonishing progress in genetics has been owing to the development of refined techniques to study microorganisms which have been found to be very useful tools in the screening of mutagenic activity of various agents and the studies aimed at quantitative aspects of such activity.

The works of Beadle and Tatum indicated that genes act through enzymes in the development of characters. For this, biochemical methods were used. Since then, a closer relationship between biochemistry and genetics has developed. It has enabled the workers to crack the genetic code. The operon concept propounded by Jacob and Monad in 1965 has helped a great deal in our understanding of gene action. The latest landmark in the progress of genetics is the development of a branch called genetic engineering which has fascinated even the layman.

In the following sections, a brief history of cytology and genetics is first traced after which the various topics included under these branches of science are dealt with.

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# 1. History of Cytology and Genetics

Every branch of science has a history. When we study a certain branch, it is first necessary to have an idea about its history. It enables us to know how it developed step by step. This knowledge helps us to gain an insight into the fundamentals of the branch. If we look at the history of cytology and genetics, it is seen that these were established recently as compared to the other main branches of science. Modern genetics was born in the beginning of the twentieth century.

Since cytology and genetics are intimately linked, their history will be dealt with together in this chapter.

#### Invention of Microscope and Beginning of Cytology

In order to study a cell, it is necessary to have a microscope. The history of cytology thus began with the invention of the microscope by Robert Hooke (England). When he cut a piece of cork into fine sections and examined one such section under his microscope, he found that it had a compartmentalised structure like that of a honeycomb. Hence, he termed the compartments observed in the cork section as cells. While describing a cell, he stated that it was bounded by a wall and contained a natural fluid. He presented his observations in the form of a paper to the Royal Society of London in 1665. This paper laid the foundation of cytology or the science of cell. After this Grew tarted the study of plants with the aid of microscope. Malpighi (Italy), who studied animal cells laid the foundation of descriptive embryology (1650-70). Both these scientists observed that the structure of plants was made up of utricles or vesicles which possessed a wall of their own. Free cells were first discovered by Leeuwenhoek (1677), a Dutch scientist. In 1677, his disciple John Ham was the first to observe spermatozoa of mammals. After this, for about a century or so, there were no noteworthy additions to the knowledge of cytology.

#### **Background of Genetics**

The beginning of the seventeenth century saw a new spirit of scientific scepticism. This was followed by actual experiments to verify statements. In 1676, Grew wrote about the

nature of ovules and pollen grains. Camararius worked on sex in plants and published a 50-page letter which convincingly proved that plants are sexual organisms. A short time before 1717, Fairchild (England) produced the first artificial plant hybrid. These discoveries of sexual organs in plants led to the establishment, mainly through the efforts of Vilmorin, of the famous French company Vilmorin-Andrieux et Cie in 1727 for seed breeding. In the following years, there was a lot of interest in plant breeding. During 1761-66, Kolreuter (Germany) published his extensive work on plant hybridization. It included the results of 136 experiments in artificial hybridization.

In England, Bakewell began experiments in 1760 on domestic animals with a view to improve their characters. After 35 years of experimentation, he concluded that inbreeding was not always harmful and was the quickest way to fix types. His experiments formed the basis of the development of many of the modern breeds of livestock. In 1773, Sprengel observed that cross-pollination occurred in plants through the agency of insects. By this period, knowledge was gained to some extent in the field of human genetics, although man is least amenable for experimentation. A British divine, Michel Lort, studied the peculiar irheritance of colour blindness and reported his findings to the Royal Society in 1779.

In 1809, Lamarck attempted to formulate a comprehensive theory of evolution. He made a very important statement that if organised parts of a thing were not made up of cell tissues, they would not have life. A more or less similar idea was expressed by Mirbel in 1802, Mayen in 1830 and Von Mohl of Germany in 1835. Darwin and Buffon, both contemporaries of Lamarck, studied in this period the various problems connected with the

development of individuals, race and species.

In 1820 the German scientist Nasse became interested in haemophilia, a disease prevalent in the royal families of Europe. He studied its inheritance and propounded the law of sex inheritance. In England, Goss (1822) was studying how characters were transferred from generation to generation. He reported dominance, recessiveness and segregation in pea hybrids but was unable to interpret them. In 1823, Knight, a plant breeder, also observed dominance, recessiveness and segregation in pea. He was the propounder of the Knight-Darwin law of cross-breeding. With the help of this law, the value of crossing to produce better plants could be determined. The term 'dominant' was first used by Sageret of France in 1826. He classified contrasting characters in the parents of a cross in pairs and cited unit characters in the human eye colour. During 1840-50, Vilmorin made many genealogical studies while carrying out breeding work in wheat, oat and sugarbeet, and developed the progeny test. In 1843, Couteur (Island of Jersey) published a summary of his work on wheat breeding. According to De Vries, this formed the basis and origin of variety testing. Methods similar to those of Couteur were also independently developed a little earlier by Sheriff (Scotland), who is credited with the production of many outstanding varieties.

The year 1859 is very important in the history of biology. It was in this year that Darwin's Origin of species was published which revolutionised the outlook of contemporary biologists. It was the turning point in biological thought, and an era of evolutionary and experimental approach began. Darwin established that plant and animal species are evolved from pre-existing ones gradually and natural selection is an important factor in the process of evolution. His theory of evolution was instrumental in disposing of the prevailing doctrine of fixity of species.

In 1863, Godron and Naudin, both from France, independently published their results on

plant hybridization. In his paper, Naudin confirmed the findings of Sageret. He also discussed the work of earlier hybridizers. He reported dominance and segregation in Datura hybrids, although he did not work with a single character at a time. He also did not make statistical analysis of his data on the second generation. In spite of these drawbacks in his work, it may be said that he was a forerunner of Mendel.

## Progress of Cytology in the Nineteenth Century

In 1833, Brown used the term 'nucleus' to define the centrally-located, darkly-stained body in the cell. The year 1835 was an important year from the point of view of progress in cytology. It was in this year that Von Mohl described the division of cells. Another important step in the progress in cytology was the pronouncement of the cell theory by Schleiden in 1838. According to this theory, all organisms are made up of cells. This theory is regarded as one of the greatest generalisations of experimental biology. Schwann (Germany), however, made it applicable to only animals. Certain assumptions made in this theory are not, however, correct. Both Schleiden and Schwann gave more importance to the cell wall than the inner fluid. They thought that new cells were formed by the method of free-cell formation. Their idea was that there was a structureless and continuous material called cytoblastome from which cells were formed by a process similar to crystallisation.

In 1840, Purkinje (Bohemia) coined the term 'protoplasm' for the cell fluid enclosed within the cell membrane, and which has been in use since then. Before him, it was Dujardon who first gave any importance to the inner contents of the cell. He called it 'sarcode'. He tried to study its chemical properties and solubility. He stated that sarcode was a completely homogeneous matter which was elastic, contractile, transparent, and gelatinous in nature. It was insoluble in water and did not show any signs of organisation. In 1840, Payen (France) and Cohn (Germany) emphasised the essential similarity between protoplasm of plant and animal cells, and regarded it as the physical basis of life. In 1841, Von Kollikar (Switzerland) proved that spermatozoa arise from the parent body and are not parasites, as was formerly believed.

It is interesting to note that in 1848 Hofmeister (Germany) had figured chromosomes as unstained bodies but he did not realise their importance and ignored them.

In 1849, Owen (England) enunciated the principle of continuity of germ plasm. This principle was further developed by Virchow, Weismann and others. Ultimately, it took shape in the form of the modern gene theory. In 1858, Virchow established conclusively a very important principle that a cell never develops de novo, i.e. a cell always develops from another cell and does not arise anew. This was indeed the basic biological principle that contributed to the completion of the cell theory. It established the theory of continuity of all life from a remote beginning, and thus dealt a final blow to the theory of spontaneous generation.

In 1861-62, Schultz and De Bary (Germany) proved that there is essential unity in all living cells. In 1865, Schweigger-Seidel and Valette, both of Germany, independently proved the unicellular nature of a spermatozoon and showed that it contains cytoplasm and a nucleus.

#### Birth of Modern Genetics

The above was the background when Mendel read his paper in 1865 in the meeting of the Brunn Natural History Society. The paper was published in the proceedings of the society in the following year. It is difficult to state how much knowledge of the work done in cytology and genetics was known to Mendel, but the fact that he was in constant touch with Nageli, the renowned biologist of his time, and was also using the library containing the works of Darwin and biological literature including the work of plant hybridization, indicates that he must have benefited by them.

The year 1866 is regarded as the year of the birth of modern genetics as it was in this year that Mendel's paper embodying the results of his hybridization experiments was published in the Proceedings of the Brinn Natural History Society (Verhandlungen der Natur Forschenden Verein in Brünn). This paper laid the scientific foundation of genetics. However, for 34 years this work remained unknown to the scientific world. It was only in 1900 that it was rediscovered simultaneously by De Vries (Holland), Correns (Germany) and Tschermak (Austria).

#### Work in Post-Mendelian Period

Chromosomes were first described in 1875 by Strasburger (Germany). In the same year, Hertwig (Germany) experimentally proved that fertilisation is brought about by the union of the spermatozoon nucleus and egg nucleus, and in this sexual process the male and female gametes have an equal share. His work indicated clearly that genetics is essentially a problem of cell physiology.

In 1882, Flemming (Germany) stated that during cell division, chromosomes split longitudinally. In 1883, Van Benedin (Belgium) discovered that when gametes are formed, the number of chromosomes is reduced to half to that present in the body cells. At about 1884-85, independent researches of Hertwig, Strasburger, Kollikar, Weismann and Flemming contributed considerably to the knowledge of cell division. In 1885, Rabl (Austria) proved the individuality of chromosomes. In the same year, Weismann published an important theory which explained the behaviour of chromosomes during meiosis and fertilisation as well as the earlier observations of Van Benedin, Strasburger, Flemming, etc. The theory predicted two kinds of cell division, mitosis and reduction, in which the chromosome number would be reduced to half by a separation of paternal and maternal chromosomes. Boveri (Germany) (1887-88) verified this theory in respect of reduction division and proved its correctness by his work on Ascaris.

In 1889, Galton (England) proposed the law of ancestral inheritance. It indicates how characters in offspring are determined and the relative influence of parents, grandparents, etc., in their inheritance. Weismann published his germ plasm theory in 1892. It helped to dispose of the prevailing theory of inheritance of acquired characters formulated by Lamarck.

In 1894, Bateson (England) laid emphasis on the study of discontinuous variations in solving the problem of heredity. This was an approach to the idea of Mendelian units of heredity. At this time, Spillman (USA) who was working on wheat had also reached almost the same conclusions as Mendel did.

In 1899, Cuenot (France) who was working on animals and Strasburger on plants put

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forth a theory that the sex is not affected by environment but is controlled within the reproductive cells.

In 1902, Montgomery (USA) showed that during meiosis, the homologous paternal and maternal chromosomes pair before actual reduction in the number of chromosomes and formation of gametes has taken place. Sutton (USA) was the first to propose the chromosome theory and in 1902 he showed parallelism of behaviour between chromosomes in meiosis and Mendelian factors an heredity. In this manner he linked cytology to genetics, and the branch of cytogenetics thus arose.

In 1904, Davenport (USA) proved that the inheritance of polydactyly was as per Mendelian laws. In the ame year, Morgan began working as a professor of zoology at the Columbia University. In collaboration with his students, he started important researches in the field of genetics. This group later on became famous as the Columbia group. Shull and East (USA) started work independently on inbreeding in maize. Their experiments helped to prepare the theoretical as well as experimental background of genetics. In 1906, Bateson coined the term 'genetics' for the science of heredity. In the same year, the American geneticists, Woodworth and Castle found that the fruitfly, Drosophila was the best experimental material for genetic research. The Morgan group used Drosophila extensively and contributed significantly to the progress of genetics. In 1906, Lock (England) suggested the possibility of a relation between chromosomes and linkage. In 1907, Correns put forward the theory that there are two types of male gametes, the male determiner and the female determiner. In the same year, Strasburger used the term 'haploid' for defining the reduced number of chromosomes and the term 'diploid' for the double number of chromosomes. It was proved by Lutz (USA) that in the gigas mutation of Oenothera lamarckjana, the chromosome number is double the number in O. lamarckiana, i.e. it is tetraploid. This work gave impetus to the study of polyploids. The Swedish geneticist Ehle worked on the inheritance of colour of wheat grains and laid the foundation of research in quantitative inheritance.

In 1909, Janssens (Belgium) put forward the theory of crossing over. In 1915, Morgan and his students, Sturtevant, Bridges and Müller published The Mechanism of Mendelian Heredity based on their researches of the genetics of Drosophila. This book is regarded as a very important step in the progress of genetics. In 1916, Shull observed hybrid vigour in F<sub>1</sub> hybrid of maize. He used the term heterosis for this vigour. This discovery stimulated rapid progress in plant hybridization.

Between 1911 and 1922, several scientists, for instance, Pearl, Collins, Kempton, Gold-schmidt, Cuenot, Gates, Jennings, etc., tackled multifold problems in genetics and contributed a great deal to its progress. In 1923, Wright (USA) published a book on the application of coefficient of inbreeding to the irregular system of inbreeding. From then on several attempts were made at using Mendelian inheritance in reproduction of organisms. The works of Wright, Fisher, Haldane and others are significant contributions in this direction.

In 1927, Müller, Stadler and Goodspeed independently observed that the rate of mutation was increased by x-rays and thus it was possible to induce mutations artificially. This was a very important discovery in the field of genetics and is regarded as a very significant step in its progress. In the same year, Hanson (USA) also realised that it was possible to induce mutation in genes by using radium. In 1934, Painter (USA) found giant chromosomes in the salivary glands of *Drosophila* and realised their genetic value in making a detailed study of the chromosome structure.

Morgan was the first to put forward the concept of the gene. He tried to visualise the size of a gene. After the Second World War, there was rapid progress in the subject of the gene concept. In this period, important researches were made using microorganisms. The contributions of Beadle, Tatum, Lederberg, Pontecarvo, Catcheside, Benzer, Khorana, Bonner and others as regards modification in the classical concept of gene are very important. Their researches brought the progress in genetics to the molecular level, and today there is a branch called molecular genetics dealing with such aspects.

After the First World War, the English scientist Wrinch attempted to solve the problem of gene action on chemical basis. Griffith's experiments on bacterial transformation made in 1928 mark the beginning of the progress in the knowledge about hereditary material itself. In 1944, Avery, MacLeod and McCarty showed that DNA (deoxyribonucleic acid) was the hereditary material. The experiment by Hershey and Chase using radioisotopes 32P and 35S confirmed it. After this significant discovery, a number of geneticists and biochemists concentrated their work on DNA.

As mentioned earlier, in the beginning of the twentieth century, it was realised that there was an intimate relationship between Mendelian inheritance on the one hand and meiosis and fertilisation on the other. This knowledge helped in understanding how specific chromosome 'groups' remain stable in species. From then on, research in cytology and genetics went hand in hand. It indicated that while studying inherited actions, cytology could not be ignored.

Let us again turn to the history of cytology. At the end of the ninetienth century, Overton proposed that the cell membrane is of a lipid nature and it is membraneous. In 1907, Harrison succeeded in culturing living nerve cells from the uterus of a frog and showed that they also differentiated. His work proved to be a guide for further researches on living cells, which later on led to the development of the tissue culture technique. The work of Carrel in 1912 on this technique had an important bearing on the progress of cytology. A further step was in the direction of microsurgery of microorganisms. This was used for studying physiochemical properties of living cells. At the beginning of the twentieth century, Schuten and Barber used a micropipette for isolating unicellular organisms. In 1911, this technique was followed by Kite in cytology.

During the Second World War and afterwards, researches in cytology were mainly of a chemical nature, and led to the development of a separate branch of science called cytochemistry or biochemical cytology. At the beginning of the twentieth century, Fisher and Hofmeister independently found that molecules of protein are made of a small number of amino acids which are bound by peptide bonds. Although Mischer succeeded in isolating nucleic acids from pus cells, spermatozoa and erythrocytes of birds, as early as 1869 and later in 1891, no attention was paid to it for a long time. It was only after Avery, MacLeod and McCarty isolated DNA in 1944 and showed that it was the hereditary substance, that scientists were attracted towards it.

Another step in the development of cytology was the concept of catalytic activity put forth by Ostwald and the discovery of cell enzymes. Ostwald showed that the energy required for cell activities is obtained through various chemical reactions catalysed by enzymes. The main types of oxidation in cells were discovered by Wieland in 1903 and by Warberg in 1908. The mechanism in this respect was explained by Kylin in 1934. His work changed the prevalent view about cytology. Since then, chemistry and physics have been increasingly used for researches on cell reactions. That cytology is also intimately connected with biochemistry was proved when Bensley and Hoerr (1934) isolated mitochondria and analysed them using chemical and physiochemical methods. It was during this period that Claude, Hoagbrom and others provided important information on the functions of mitochondria. The progress of modern cytology has been owing to researches on this line using microchemical techniques of analysis. It has now been possible, thanks to electron microscopy, to isolate single cells and their parts. Cell biologists are also increasingly using autoradiography in their work. The close association of cytology and biochemistry led to the formation of a separate branch called molecular biology.

We have yet no complete knowledge about the cellular base of the mechanism of protein synthesis. Considerable work has been done on the translation of information stored in the DNA to the remaining part of a cell. Caspersson (1950) believed that nucleic acids produced proteins which are first transferred to the nucleolus in the form of histones and then to the cytoplasm. Lablond and Amano (1962) concluded that ribouncleic acid (RNA) and proteins are continuously synthesised in the nucleus, most probably in the chromosomal matter. Jones observed ribosome bodies in the nucleolus of erythroblast.

The idea that RNA plays a dominant role in protein synthesis was first put forward by Brachet in 1941. In the same year, Caspersson also expressed a similar thought. In 1952, Palade discovered cytoplasmic ribosomes using electron microscopy and employing the differential centrifugation method. His researches showed clearly that in the living system, nucleic acids and protein occupy a key position. In 1953, Watson and Crick suggested a model for DNA, which soon received experimental proofs in its favour. Researches thereafter have indicated that DNA has no direct role in protein synthesis and that it only acts as a template for producing RNA which directly participates in protein synthesis. In 1961, Nirenberg and Mathaei succeeded to some extent in cracking the genetic code necessary for protein synthesis. Research was also carried out on this problem by Lengyel, Speyer and Ochoa (1961) and it brought to light the fact that when RNA units are added, amino acids are incorporated. The works of Nirenberg, Ochoa, Khorana and their associates have helped to a large extent in getting more information on the genetic code. Khorana even succeeded in synthesising a gene in vitro.

The idea that genes act through enzymes was first put forward by Beadle and Tatum through their experiments on *Neurospora*. As the work on protein synthesis in cells progressed further, it become clear how genes act through enzymes. The latter being proteins, their synthesis is controlled by DNA. In this respect, the operon concept proposed by Jacob and Monad of France (1965) has been a very important step in understanding gene action.

In the 1930s, two general models were proposed for accounting crossing over. These were (i) breakage and rejoining and (ii) copy choice. Meselson and Wiegle made an experiment to distinguish between breakage-rejoining and copy choice. They used phage DNA and succeeded in getting recombinant DNA. Since then, many geneticists have concentrated on recombinant DNA to produce desired characters in plants and animals. Their researches have led to the establishment of a new branch of science called genetic engineering.

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#### SUMMARY

1. The history of cytology began with the observation of cork cells under a microscope by Robert Hooke of England in 1665.

2. Commendable progress in cytology was made in the nineteenth century. Robert Brown, von Mohl, Schleiden, Schwann, Purkinge, Payen, Cohn, Virchow, Strasburger, Hertwig, Flemming, Benedin, Kollikar and Weismann contributed immensely to the progress of cytology.

3. Genetics is the science of heredity. The foundation of modern genetics was laid by

Gregor Mendel, who is regarded as the Father of Modern Genetics.

4. The discovery of chromosomes in 1875 and information on nucleic acids, the work on the isolation of the latter and the model of DNA proposed by Watson and Crick considerably helped in the progress of genetics.

5. In the beginning of the twentieth century, a close link between cytology and genetics

was discovered leading to the branch of science known as cytogenetics.

6. The discovery by Müller and Stadler in 1927 that mutations can be induced by x-rays was a very important step in the progress of genetics.

7. In the post-World War II period the use of microorganisms as the genetic tool and

refined biochemical techniques brought genetics to the molecular level.

8. The discovery in 1961 of recombinant DNA by Meselson and Wiegle led to the establishment of a new branch of science called genetic engineering. AUS 18 OF CHARGE COME ST 18 10 31 18 19

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# 2. Structure and Characteristics of Cells

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#### Cell Concept

A cell is usually described as a structure with a prominent dense body in the centre called the nucleus and surrounded by a fluid mass called the cytoplasm. Nucleoplasm, i.e. the matter which the nucleus is made up of, and cytoplasm together constitute the living matter known as protoplasm. The difference between the cell of an animal and that of a plant is that the former is surrounded by a membrane while the latter has a cell wall. The nucleus is also limited by the nuclear membrane. The above definition of the cell does not, however, give a proper idea of the cell content. In fact, a cell is an integrated and constantly changing system. When its ability to change comes to an end, biologically it cannot remain as a cell. It is then only a mass of decomposing matter. Like the cell, protoplasm is also a system and hence, it is not possible to define it on the basis of its chemical constituents. Besides, even though it has been possible to analyse protoplasm and determine the propor tions of its chemical constituents, it has not yet been possible to synthesise a cell by combining them (Fox, 1960). We have yet no complete information about the cell system. Red blood corpuscles (RBC) do not possess a nucleus while some other types of cells are multinucleate. In general, it may be said that both the cytoplasm and the nucleus are essential parts of a system and they interact with each other. Without the nucleus a protoplast is either unable to carry on life processes or carries them for a very short time only. The same is applicable to the nucleus when removed from the cytoplasm. The physicochemical reactions that are taking place continuously in the cell are collectively called metabolism. The metabolic reactions requiring energy to synthesise complex substances from simple substances are included under anabolism, while those breaking complex substances into simpler ones and usually releasing energy are grouped under catabolism.

#### Unit of Life

A cell is the basic unit of life. It is a physical entity. It has now become possible to obtain a cell extract, analyse its constituents and study them in detail as physicists study various parts of an atom by splitting it. In some cases, the cell extract may remain active for some time, for example, it may take in oxygen, cause fermentation or form molecules. But these individual actions do not constitute life.

Loewy and Siekewitz (1969) have defined cell as a unit of biological activity bound by a membrane which is selectively permeable to substances and independently capable of self-production. The foregoing discussion indicates that it is very difficult to give a comprehensive definition of a cell. However, it is possible to give a general description of the majority of units of an organism at the cellular level.

Though a virus is smaller than a cell, it cannot survive outside the cell, where it is parasitic. A cell is larger than a molecule. It has a boundary within which chemical reactions are going on continuously. When the activity of the cell is stopped, it dies. Hence, a cytologist wants to know about cells, their organisation, and their structure from the point of view of function. The important thing to be borne in mind is that a cell is not just a complete entity but is also an elaborate organ. Whether it is in the form of a unicellular organism or a part of a tissue of a multicellular organism, it is a structural and functional unit of life.

#### Size and Form

The units of measurement used in the study of cytology are the micron ( $\mu$ ) and angstrom (Å). The former is 1/1000 of a millimetre, while the latter is  $0.001~\mu$ . For studying cell and its contents under light microscopy, the unit of measurement is usually the micron, while for ultra-microscopy the unit of measurement is the angstrom.

#### **Unicellular Organisms**

The smallest cell represented by free-living organisms is the *mycoplasma*. Its diameter is only 1000 Å. Unicellular mycoplasma organisms have been isolated from the soil. They infect certain animals and are usually referred to as PPLO (pleuropneumonia-like organisms) (Fig. 2.1). The microorganisms next in order are bacteria which are also unicellular. Bacteria are of different shapes: round (cocci), rod-shaped (bacilli) and those like screw threads (spirilla). Fibrillar bacteria resemble threads. Cocci bacteria have a diameter of about 5000 Å, while fibrillar bacteria are found even up to 20  $\mu$  long. Some of the blue-green algae are also unicellular, their diameter being  $10 \mu$ , which is slightly larger than a human RBC, which has a diameter of 7-8  $\mu$ .

Many unicellular organisms are motile, for example, flagellates and ciliates. They are also able to make movements with the help of their flagella or cilia which are cytoplasmic in nature, e.g. Euglena (which is a unicellular alga) with a flagellum. This genus has many species and one of them is with the largest cell (500  $\mu$  in length). Euplotes is ciliate and it occurs in ponds, its length being 120  $\mu$ . Diatom is also a unicellular alga with a length of 200  $\mu$  or more. Among the unicellular organisms, one of the biggest is Amoeba, its length being 1 mm (1000  $\mu$ ).

#### Multicellular Organisms

Multicellular organisms have definite tissue systems composed of cells of size mostly  $20-30~\mu$ . Among animals, the largest cell is the egg of an ostrich. Even the diameter of its yolk measures 5 cm. If we compare it with the human egg, the latter is found to be

much smaller, with a diameter of only 200  $\mu$ , while the head of a spermatozoon is just 5  $\mu$  and the tail 30-50  $\mu$ . Among plants, the largest cell is the ovule of Cycas.

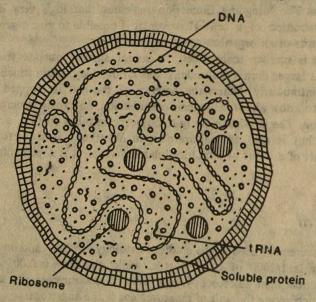


Fig. 2.1 Pleuropneumonia-like organism.

#### Cell Size

The lowest limit of the cell size is determined by the minimum number of essential constituents that can be accommo dated within a cell for it to perform its functions independently. The size of some cellular macromolecules is almost similar to that of mycoplasma. The maximum limit of the cell size is determined by several factors. Many activities in the cytoplasm are regulated and directed by the nucleus. Hence, the relation between the volume of cytoplasm and the nucleus is important. There is a limit to the volume of cytoplasm which can be controlled by the nucleus. Generally, the cytonuclear ratio in the mature cell of a given tissue is stable. Besides, diffusion of materials from one cell to another is also an important factor. The cell and its surrounding environment are also related to size and volume. If a cell becomes larger in size, the ratio between its volume and surface area becomes less thus making it difficult for the cell to be in communication with the surrounding environment. In case of some cells, it is possible to increase the surface area with long and slender protuberances or it can be reduced by introvening the outer surface.

Cells possess various shapes (Fig. 2.2). They may be elliptical, spindle-shaped, block-shaped, polygonal, columnar, discoidal, flat or plate-like. Differences in size depend mostly upon external factors such as mechanical pressure and surface tension. If a naked protoplast is left to itself, the tendency will be to assume a round shape. Besides, shape and function are also closely related, but our knowledge in this respect is meagre. In

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plants, for conduction of water and transportation of food materials, columnar or tubular cells are useful. Hence, cells of vascular tissues consisting of tracheids and vessels are columnar.

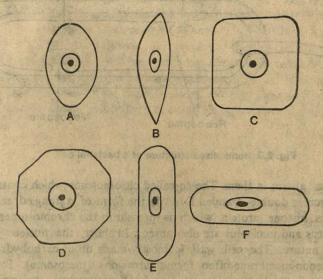


Fig. 2.2 Cell shape. A – Elongated; B – Spindle-shaped; C – Block-shaped; D – Polygonal; E – Columnar; F – Plate-like

#### Cell Structure

In PPLO and blue-green algal cells, there is no differentiation of protoplasm into cytoplasm and nucleus. The structure of their cells is also very simple.

The outer surface of PPLO (Fig. 2.1) is two-layered and made of phospholipid proteins. Inside it there are double strands of DNA. In addition, there are present ribosomes with soluble- or transfer-ribonucleic acid (s-RNA or t-RNA). With their help, the cell is able to carry out its various activities, e.g. protein synthesis. In the bacterial cell (Fig. 2.3), the nuclear region is somewhat distinct but there are no distinct chromosomes as such.

If cells of higher plants and animals in living or fixed conditions are studied, it is seen that the nuclear materials are surrounded by a definite membrane. This is called the nuclear membrane. Hence, the cellular organisation is divided into two basic classes: (i) Prokaryota and (ii) Eukaryota. Organisms included under the former class are called prokaryotes, while those belonging to the latter class are known as eukaryotes.

#### Prokaryotes

Bacteria or blue-green algal cells come under the class prokaryota. These are small cells  $(0.5-3.0~\mu$  in diameter) without a nuclear membrane. Nucleoli and cell organelles such as mitochondria, chloroplasts, Golgi complex and lysosomes which are limited with a distinct

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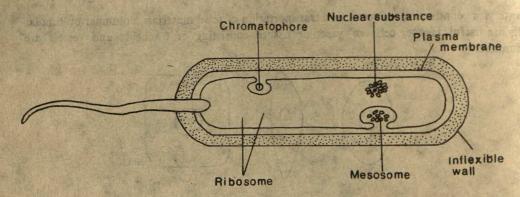


Fig. 2.3 Generalised structure of a bacterial cell.

membrane are also absent in them. The so-called chromosome which contains the genetic information is a circular double-stranded DNA in the form of a tangled mass (nucleoid). It does not possess histone protein which is present in the chromosomes of eukaryotes. The mitotic apparatus and nucleolus are also absent. In short, the nucleus of prokaryotes is of a primitive nature. The cell wall is mostly made up of carbohydrates and amino acids. The protoplasmic membrane often forms intrusions (mesosome). Prokaryotic cells do not exhibit cytoplasmic streaming or amoeboid movements. Locomotion is often achieved by simple fiagella which do not possess the multistranded complex structure of the eukaryotes. Their distribution is universal as they grow very fast. In their biochemical reactions, they show versatility and there is genetic flexibility in their heredity.

Electron micrograph studies of blue-green algal cells have now revealed considerable information about their structure (Fig. 2.4). As has already been stated, these cells do not have a nuclear membrane, i.e. there is no well-organised nucleus. The protoplast is differentiated very slightly into a more densely-coloured area at the periphery, while the central area is lightly coloured. Some species exhibit a relatively dense mass of the material (usually regarded as the nuclear material) at the centre. It is called the central body. There is some staining differentiation of the central body similar to that of chromatin of the nucleus of higher plants. It has been shown that chromatin consists of DNA, RNA and protein. It seems that the DNA molecule determines the structure and heredity of the cell.

Electron microscope study (Pankratz and Bowen, 1965) of the structure of the cell of the blue-green filamentous alga Symploca muscorum belonging to the Oscillatoriaceae (Fig. 2.4) shows that it is surrounded by a mucilaginous sheath consisting of three layers of fibrillar material. There is localised thickening in certain areas. During cell division new cross-walls are formed which grow inwards from these areas. The plasma membrane is elaborated inwardly, especially, the ones in contact with developing cross-walls. Lamellae are also present and are concentrated more near the peripheral region of the cell. They are elongated, flattened sacs apparently containing chrolophyll. Structural granules occur near the mature walls and are called cyanophycin particles. According to some research workers, these particles are equivalent to mitochondria. Polyhedral bodies are present in association with the genetic material but their composition and function are unknown. The nucleoplasm consists of fibrillar material and staining reactions indicate the occurrence of DNA in these

fibrils. The ribosomes are seen as tiny dense granules. Although they are dispersed throughout the cell, they are most closely associated with fibrillar nucleoplasm. Granules occur between the lamellae and are said to be concerned with certain metabolic functions connected with respiration or photosynthesis or they may be in the nature of reserve food. There are also vacuole-like inclusions in which lipids may be present. Their function is unknown. Pankratz and Bowen have reported the presence of cylindrical bodies in the cells of Symploca muscorum, although these have not been found in the cells of other blue-green algae. One reason for this could be that they are perhaps intercellular parasites or symbionts (Ditmer, 1968). Phycocyanin, responsible for the blue-green column, tends to be accumulated in the peripheral area. Besides these, chlorophyll a, carotinoids, and a red pigment could also be present in the cells.

#### Eukaryotes

The characteristic feature of cells of this group is the presence of a true nucleus bound by a membrane. The cell is therefore differentiated into two distinct parts: cytoplasm and the nucleus. All plants and animals come under this group except blue-green algae and bacteria. The cytoplasm is amorphous and homogeneous substance containing refractile particles of different sizes, prominent among them being mitochondria. The peripheral layer called the ectoplasm is often relatively more rigid and non-granular. It usually acts as a colloid and undergoes reversible gelation and solation. Such a transformation is seen in amoebae during the extension of pseudopodia. The internal cytoplasm, called the endoplasm, is less viscous and contains different kinds of granules. The membrane system of eukaryotic cells is extensive, e.g., endoplasmic reticulum and Golgi complex. The organelles (e.g., nucleolus, mitochondria, chloroplasts, lysosomes, etc.) also have their limiting membranes. The nucleus is distinct and definite and contains the nucleolus and chromosomes which are made of DNA and histone. The cells of eukaryotes not only perform functions carried by prokaryotes but are also capable of acting as the constituents in the life processes of extremely differentiated organisms (Fig. 2.5).

An important difference between plant and animal cells (Figs. 2.6 and 2.7) is that the former possesses a cell wall, which is a protective thick covering on the cell membrane. For communicating with neighbouring cells, minute protoplasmic strands called plasmodesmata pass through the cell walls. In animal cells, the plasmodesmata are covered with a thin layer which is, in fact, an extra cover of the cell membrane.

A living cell is usually studied under a light microscope. It has not yet become possible to study it under an electron microscope as it can only be studied in the fixed condition. Our present knowledge about cell and its organelles is, however, mostly from the study of fixed cells. Detailed information in this respect is given in the next chapter.

Majority of cells cannot be observed with the naked eye, because its resolving power is only about 0.1 mm. Objects of lesser dimensions than this cannot be either seen by our eyes or if seen, they appear indistinct. Minute objects look larger through a light microscope (Fig. 2.8A) because they are magnified. The resolving power of a microscope depends upon the thing which illuminates the object. If the object is nearer than half length of the illuminating light wave, it will not be seen distinctly through the light microscope. In other words, even if a very good microscope with an oil immersion lens is used and the wavelength

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of light is 5500 Å, two discrete points separated by a distance less than 2700 Å  $(0.27 \,\mu)$  will not be resolved. It is because of this reason that before the advent of electron microscopy, one did not know of the existence of several minute parts of cells.

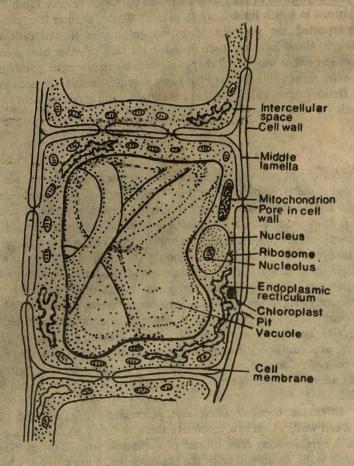


Fig. 2.6 A typical plant cell.

#### **Electron Microscope**

It has now become possible to examine very minute objects under an electron microscope (Fig. 2.8B). In this microscope, very fast neutrons are used in place of light wave. When electrons penetrate the specimen which is being viewed, parts of the cell either absorb them or they are differentially scattered. After passing electrons through lenses, an image of the specimen is formed on the electron-sensitive photographic plate or on the fluorescent screen. Because our eye is not stimulated by electrons there is the necessity of a plate or screen. In an electron microscope, the optical arrangement is like that in a light microscope. The only

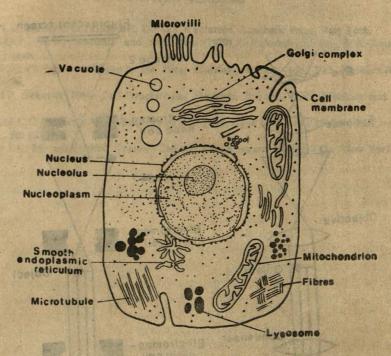


Fig. 2.7 A generalised animal cell, (Redrawn after Loewy and Siekevitz, Cell Structure and Function, 1969.)

difference is that instead of glass or quartz lenses, magnetic coils are used for illumination. When electrons are accelerated at a 5000-V potential difference, their wavelength is about 0.05 Å, i.e. it is 105 times the average wavelength of white light. This means that an electron microscope is theoretically able to resolve those objects that are separated by a distance of 0.05 Å or half of 0.025 Å. This distance is, of course, less than the diameter of an atom. But actually, the resolving power of an electron microscope is 2 to 3 Å. Although it is not possible to identify items at this level, we can easily observe biologically important macromolecules. In other words, an object seen with the naked eye is magnified 100,000 times by an electron microscope. It is because of this quality that cytology has made tremendous progress in recent years. It has enabled us to get detailed information on the cell structure.

#### SUMMARY

1. A cell is an integrated and constantly changing system. It is the basic unit of life and a physical entity.

2. The smallest cell is the mycoplasma (PPLO). The size of some cellular macromolecules is almost similar to a mycoplasma.

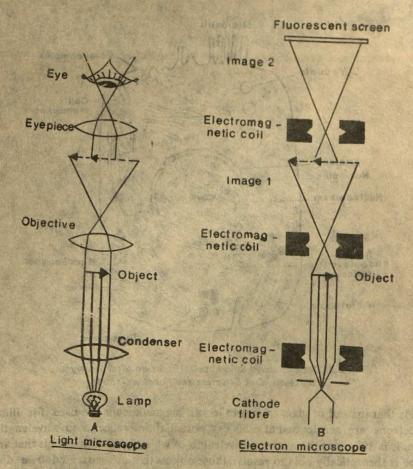


Fig. 2.8 Diagrammatic representation of electron and light microscopes.

- 3. Cells possess various shapes.
- 4. The structure of the PPLO and blue-green algal cells is very simple. There is no differentiation of protoplasm into cyptoplasm and nucleus. In a bacterial cell, the nuclear region is somewhat distinct but there are no distinct chromosomes. All these organisms are included under the class prokaryota. A definite nucleus is organised in the cells of higher plants and animals. As such, they come under the class eukaryota.
- 5. As the resolving power of the electron microscope is on an increasing scale, itis now possible to gain detailed information on the cell structure.

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# 3. Plasma Membrane (Cell Membrane)

Cytoplasm comprises the outermost surrounding plasma membrane and the ground substance which may include organelles and various types of metaplastic bodies like secretion granules, yolk, globules, etc. This ground substance is called the ground matrix or hyaloplasm. The outermost part of the protoplasm in plant cells is called plasmalemma because it is similar to plasma membrane in nature.

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The plasma membrane is a definite protoplasmic membrane surrounding the cell cytoplasm. Plant cytologists prefer to use the term 'cell membrane' to denote this membrane. In 1931, Plows introduced the term plasmalemma to denote cell membrane as he thought the latter term might be confused with the term 'cell wall'. It is usually stated that the cell membrane consists of plasmalemma along with the surrounding cell cement.

The existence of a plasma membrane in a living cell can be proved indirectly by microsurgical experiments. If you put a cell which is not permeable to a dye in a medium injected with the dye, it becomes coloured. You will also observe that the dye remains within the limits of the plasma membrane. Puncturing of a cell by means of a microneedle causes injury to the plasma membrane. It can, however, be repaired within certain limits. But if the injury is severe, and there is absence of calcium ions, the cytoplasm will flow outwards, with the result that the cell will die.

Several methods have been used to isolate plasma membrane from a variety of cells, e.g. sea urchin's eggs, liver cells, etc. It is easily separated from erythrocytes subjected to haemolysis. It may be possible to remove haemoglobin completely at pH 8 but it is likely that other components may also be extracted along with it. Hence, plasma membrane separated in this way is not a true membrane, but a ghost membrane, according to several research workers.

The plasma membrane is selectively permeable. Penetration of substances through the plasma membrane seems to result from simple diffusion. The rate of penetration depends upon the metabolism of the cell. If a substance is quickly utilised in the metabolism, there is acceleration in its penetration which is also facilitated by the presence of specific enzymes in the membrane itself. For example, the presence of phosphatase enzyme in the plasma membrane of yeast cells facilitates penetration of phosphates and glucose.

The plasma membrane is capable of making its surface small or large according to the needs of the cell. It can be extended by producing long and slender processes called *microvilli* (Fig. 3.1). These are found in cells which are active in the transport of substances, for example, abortive cells in the intestinal epithelium or secretory cells. The plasma membrane

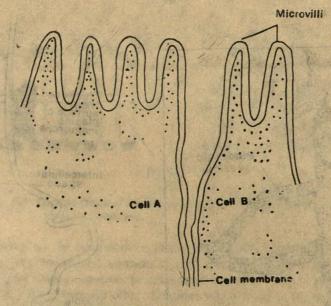


Fig. 3.1

also causes transitory evagination of the surface of the cell. These are the ways which lead to the extension of the cell surface. If some liquid is to be entrapped, protrusions coalesce and the liquid enters into the form of vacuoles or vesicles (Fig. 2.7). Such a type of transport involving, as if, "bulk drinking" of the external solution, is termed pinocytosis (Fig. 3.2).

In various cell types, localised thickenings of closely apposed cell membranes take place. The thickened parts of each membrane usually lie very close to each other and hence appear as a single structure known as desmosome (Fig. 3.3). These contiguous regions form a kind of bridge between neighbouring cells of many tissues.

# Membrane Models

Various models have been proposed to explain the physical and biological features of the cell membrane. They can be divided into two groups; the bilayer model group and the micellar or subunit model group. In the first group, the protein and lipid constituting the membrane are supposed to occur in layers, while in the second group the existence of a number of similar subunits has been envisaged. Some of these are described briefly below.

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#### Bilayer Models

#### 1. Lipid membrane

It was observed by Overton (1899) that fat-soluble substances passed easily through the cell membrane from which he concluded that it contained lipids. The works of Hober (1910) and

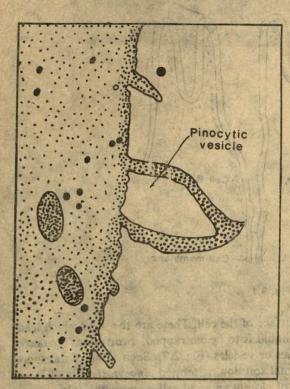


Fig. 3,2 Extensions of the plasma membrane of leucocyte (diagrammatic). (Redrawn from Loewy and Siekevitz, Cell Structure and Function, 1969.)

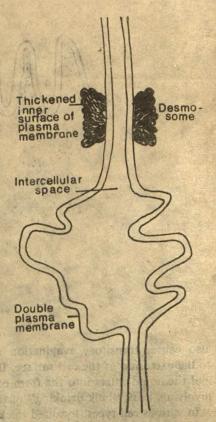


Fig. 3.3 Part of plasma membrane showing desmosome and thickened inner surface.

Fricke (1925) also suggested the existence of a lipid layer. Gorter and Grendel (1925) observed that the surface membrane of erythrocytes was made up of a double layer of lipid molecules. According to them, the polar groups of these molecules were present on the outside of the layers.

#### 2. Danielli-Dawson model

Studies by Harvey and Cole (1931) and Danielli-Dawson and Danielli (1935) on the surface tension of cells indicated the presence of proteins in the cell membrane (Fig. 3.4). Dawson and Danielli (1937) therefore proposed a lipoprotein model. It consists of a bimolecular lipid layer as conceived by Gorter and Grendel. There are two layers of molecules with their polar regions on the outer side. It is believed that globular proteins are associated with the polar groups of the lipid.

The Danielli-Dawson model eventually underwent several modifications. In 1938, Danielli stated that the proteins in contact with the lipid were arranged tangentially while globular

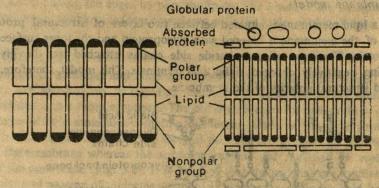


Fig. 3.4 Danielli-Dawson model depicting the trilaminar sandwich nature of plasma membrane.

proteins were present on the outer surface. David and Danielli (1943) and Danielli (1954) further modified the model and considered that the proteins are in the form of B-chains (Fig. 3.5). According to one variation, proteins are believed to be in a coiled form on both sides of the lipid layer. There are other variations which visualise the existence of globular proteins on both the surfaces and helical proteins extending into the pores.

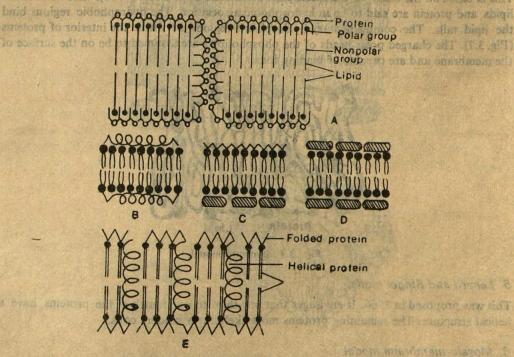


Fig. 3.5 A-Danielli model of plasma membrane with proteinlined pores. B-E-Other suggested arrangements of proteins.

#### 3. Greater membrane model

In this model, a lipid membrane is situated between two layers of structural proteins. The outer surface of the membrane is covered with glycoprotein and the inner surface with unconjugated protein. There are oligosaccharide side chains attached to the glycoprotein (Fig. 3.6) having negatively charged sialic acid terminals. This model, therefore, indicates asymmetry and structural polarity of the membrane.

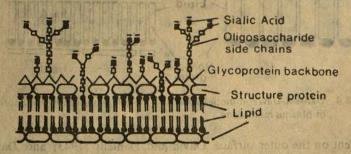


Fig. 3.6 Greater membrane model.

Fig. 3.5). According to one variation, proteins are believed to be in

#### 4. Benson's model

This is based on the study on the chloroplast membrane by Benson (1966). The membrane lipids and protein are said to be in hydrophobic association. The hydrophobic regions bind the lipid tails. The complementary hydrophobic regions are within the interior of proteins (Fig. 3.7). The charged polar heads of the phospholipids are assumed to be on the surface of the membrane and are capable of binding ions.

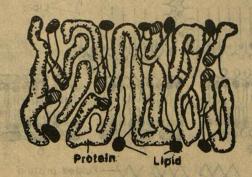


Fig. 3.7 Benson's model.

# 5. Lenard and Singer model

This was proposed in 1966. It envisages that one-third to one-fourth of the proteins have a helical structure. The remaining proteins most likely form random coils.

#### 6. Mosaic-membrane model

Baum (1967) proposed a model in which the backbone of the membrane consists of cuboidal units 80 A in diameter. Each unit of protein is considered to be toast-shaped. It is covered

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on both sides and along the edges by phospholipids. The heads of the phospholipids are free whereas their hydrophobic tails form complexes with the hydrophobic surface of the protein.

#### 7. Fluid-mosaic model

This was proposed by Singer and Nicholson in 1972. According to this model, the biological membranes are assumed to be qualified structures in which the arrangement of lipids and integral proteins is in a mosaic pattern. The cell membrane of a eukaryote is supposed to be a double layer of lipid molecules containing different kinds of globular protein molecules and sterols. The polar heads of the integral proteins are regarded as protruding from the surface of the membrane whereas the nonpolar regions are regarded as embedded in the interior of the membrane (Fig. 3.8). It is believed that the proteins are involved in the enzymatic activity and passing of molecules across the membrane while the lipid layer acts as the permeability barrier.

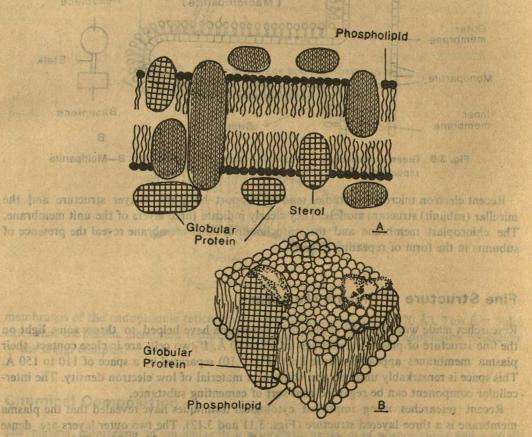


Fig. 3.8 Fluid-mosaic model of Singer and Nichlson. A—Schematic section through eukaryotic fluid mosaic membrane, B-Bacterial membrane, model angle membrane

#### Micellar Models

According to Fernandez-Moran (1962) and Sjostrand (1963), the cell membrane consists of globular units or elementary particles which are closely packed and are repeated. They do not, however, consider that structure is present in all membranes. They admit the possibility of the presence of both the unit membrane and globular form in the cell.

Green (1970) considers the cell membrane as consisting of fused repeating units. He recognises two kinds of membranes, monopartite and multipartite (Fig. 3.9). In the former, projections are absent in the repeating units, e.g. plasma membrane of erythrocytes, while in the latter, projections are present. They include macrotripartite (larger) and microtripartite (smaller) units. These occur in membranes in which electrons transfer is coupled with ATP synthesis. ATPase function is localised in the head pieces of the macrotripartite membrane while localisation in the case of the micropartite membrane is in the base pieces.

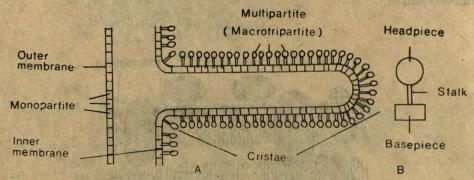


Fig. 3.9 Green's repeating unit model. A—Crista of mitochondrion. B—Multipartite repeating unit.

Recent electron microscope studies tend to support both the bilayer structure and the micellar (subunit) structure models. They clearly indicate three layers of the unit membrane. The chloroplast membrane and the mitochondrial inner membrane reveal the presence of subunits in the form of repeating globules.

# Fine Structure of Plasma Membrane

Researches made with the help of electron microscope have helped to throw some light on the fine structure of plasma membranes of 60 to 80 Å. If two cells are in close contact, their plasma membranes appear as dense lines (Fig. 3.10) separated by a space of 110 to 150 Å. This space is remarkably uniform and contains a material of low electron density. The intercellular component can be regarded as a sort of cementing substance.

Recent researches using improved cytological techniques have revealed that the plasma membrane is a three-layered structure (Figs. 3.11 and 3.12). The two outer layers are dense and have thickness of about 20 Å. The middle layer is about 35 Å thick. This structure is called the *unit membrane*. It is found in most intracellular membranes. The thickness of the unit membrane (about 100 Å) is more than that of the plasma membrane, the intracellular

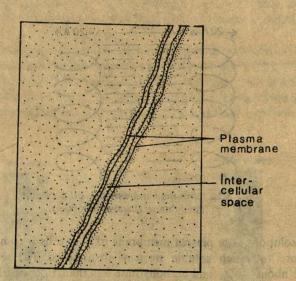


Fig. 3.10 Plasma membrane boundary between two cells. (Redrawn from Loewy and Siekevitz, Cell Structure and Function, 1969.)

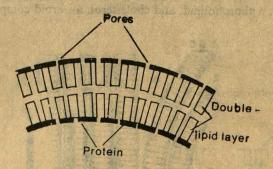


Fig. 3.11 Plasma membrane.

membranes of the endoplasmic reticulum and the Golgi complex (50-70 Å). This fact indicates that the plasma membrane is different from the membranes of the endoplasmic reticulum or the Golgi complex.

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# Chemical Composition of the comp

The plasma membrane of animal cells is principally made up of protein, lipid and a small amount of carbohydrate (Figs. 3.13 and 3.14). The outer surface is negatively charged as it is associated with carboxyl and phosphate groups and sialic acid. Hence, it becomes possible for the plasma membrane to bind easily the positively-charged proteins which can be removed by

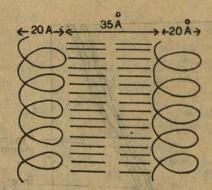


Fig. 3.12 Unit membrane model of a plasma membrane (Sandwich model).

washing in saline solutions. The plasma membrane of liver cells is composed of phospholipoglycoprotein core to which soluble proteins are attached. The percentage of protein is about 60%, lipid about 40%, and carbohydrate less than 1 % of the total dry weight. Three different classes of protein are present in the membrane. These are structural proteins, enzymes and carrier proteins. Structural proteins constitute the backbone of the cell membrane. Carrier proteins or permease are concerned with the transport of substances across the membrane against a concentrated gradient. The chief lipid components of the plasma membrane are lecithin, a phospholipid, and cholesterol, a steroid compound. Carbohydrates

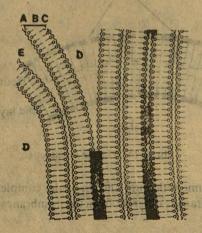


Fig. 3.13 Molecular structure of unit membrane forming a myelin sheath, A—Protein and carbohydrate layer on outer side of the membrane. B—Lipid core.

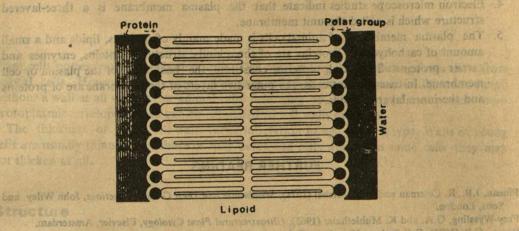
C—Protein layer of inner side.

D—Cytoplasm of Schwann cell.

E—Extra-cellular matter. (Redrawn after Robertson, Scientific American, Vol. 206, 1962.)

are in the form of polysaccharides and distributed between lipids and proteins. The mechanical capacity of the plasma membrane to expand and contract might be due to fibrous protein. , embanes as anesderine omessicono umitab a a anadmam amasic of

Most of the work on plasma membrane pertains to animal cells and no detailed information is available in respect of the plasma membrane of plant cells. The structure of the plasma membrane of plant cells is also three-layered. The outer two layers are of protein and the inner layer consists of lipid. The structure of their plasma membrane depends upon synthesis of substances required for cell-wall formation and their transport.



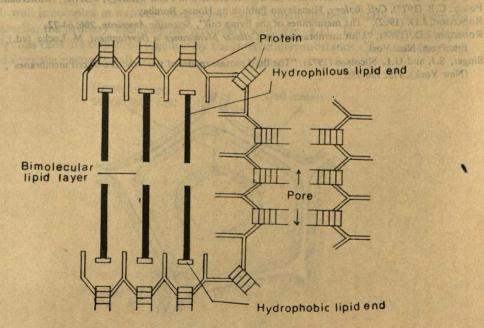


Fig. 3.14 Plasma membrane of animal cells.

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- mechanical capacity of the plasma membrdge. 1. The plasma membrane is a definite protoplasmic membrane surrounding the cell cytoplasm. It is usually referred to as the cell membrane by plant cytologists.
- 2. The plasma membrane is selectively permeable and is capable of making its surface less or more according to the cell needs. In various cell types, localised thickenings of closely apposed cell membranes take place. 19 self benefit to state no recell terms out but
- 3. Various models to explain the physical and biological features of the cell membrane, have been proposed. They come under the categories of bilayer and micellar models.
- 4. Electron microscope studies indicate that the plasma membrane is a three-layered structure which is called the unit membrane.
- 5. The plasma membrane of animal cells is made mainly of proteins, lipids and a small amount of carbohydrate. Proteins are of three kinds; structural proteins, enzymes and carrier proteins. Structural proteins constitute the backbone of the plasma or cell membrane. In case of plant cells, the outer two layers of the membrane are of proteins and the inner layer of lipids.

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# 4. Cell Wall so mand to the second of the se

Plant cells possess non-protoplasmic walls. This outstanding feature distinguishes them from animal cells. However, there are a few plant cells (e.g., motile spores in algae and fungi) without a wall at all while a few animal cells belonging to lower organisms possess non-protoplasmic envelopes which can be compared to cell walls of plants.

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The thickness of plant cell walls varies in relation to age and cell type. Walls of young cells are usually thinner than those of fully-developed cells whereas in some cells they may not thicken at all.

#### Structure

The cell wall is complex in nature. Three layers can be distinguished clearly. These are the middle lamella, the primary wall, and the secondary wall (Fig. 4.1). The primary walls of two contiguous cells are cemented together by the intercellular substance. The secondary wall is formed over the primary wall.

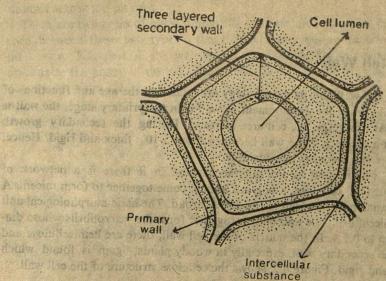


Fig. 4.1 Diagram showing layers of a cell wall.

#### Middle Lamella

This develops between adjacent cell walls during cell division and serves as a cementing material between the primary walls of the adjacent cells. It is difficult to identify the intercellular substance in mature tissues. The distinction between the intercellular lamella and the primary wall is often lost during the extension growth of the cell. Kerr and Bailey (1934) and Esau (1953) use the term 'middle lamella' only with reference to the intercellular substance. The middle lamella is amorphous, colloidal and optically inactive. It consists of pectin, cellulose, calcium and polymers of different kinds.

#### **Primary Wall**

This is formed during the early stages of growth and development of the cell. In many kinds of cells, it is the only wall. It is composed of cellulose and pectic compounds and also usually contains variable amounts of non-cellulosic polysaccharides and hemicelluloses. It may become lignified. It is optically anisotropic and elastic, and undergoes extension with the cell growth. When it is thick, it frequently exhibits conspicuous lamination which is indicative of growth in thickness due to a successive deposition of layers. Primary walls are usually accompanied by living protoplasm. Therefore the walls of dividing and growing meristematic cells and of those which retain living protoplasm are primary.

#### Secondary Wall

This is laid down on the primary wall after the cell has ceased to enlarge. The cells possessing secondary walls are frequently without protoplasts at maturity, e.g. tracheids, vessel elements, and certain fibres. Secondary walls are most characteristic of highly specialised cells. They usually show three layers: the outer, the middle and the inner layer (Fig. 4.1). Their principal constituents are cellulose, hemicellulose and lignin. Their main function is mechanical.

## **Organisation of Cell Wall**

The organisation of the cell wall and its morphology are related to the age and function of the cell. In young cells, when the development of wall is in the primary stage, the wall is elastic and 1-3  $\mu$  thick. At this stage, the cell area increases. During the secondary growth of the cell wall, expansion ceases and the wall becomes about 5-10  $\mu$  thick and rigid. Hence, the tensile strength of the cell increases considerably.

The matrix of the cell wall is composed of polysaccharides. In it there is a network of microfibrils. Many chains of cellulose, a polysaccharide, come together to form micelli. A group of micelli forms a microfibril which is 100-150 Å broad. The basic morphological unit of the cell wall is the microfibril. The microfibrils together form the macrofibrils whose diameter may be even up to  $0.5 \mu$ . In the matrix of the cell wall, there are hemicellulose and pectic substances. In the secondary wall, especially in woody plants, lignin is found which makes the wall strong and rigid. Figure 4.2, shows the cellulose structure of the cell wall.

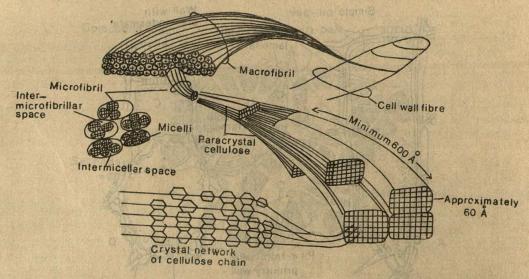


Fig. 4.2 Cellulose structure of the cell wall.

#### Pits

Depressions of cavities called pits are usually present in the cell wall. Since the pits in the primary cell wall and the secondary cell wall differ in their structures, the pits of the former are termed primary pit fields and the latter just pits (Committee on Nomenclature, 1933).

The primary pit fields of a meristematic cell may be deep and numerous so that the wall shows a beaded appearance in a sectional view (Fig. 4.3). They may be slightly modified as in some types of parenchyma cells or considerably modified as in case of specialised cells such as sieve elements. The primary wall in the primary pit field is relatively thin and continuous across the pit area.

There are two types of pits—simple pits and bordered pits. The most fundamental difference between these two types is that the secondary wall in the bordered pit arches over the pit cavity. This part of the wall forms the border. The secondary wall narrows down its opening to the lumen of the cell. Therefore if the pit is viewed from the side, it looks like a dome opening at the top. The part of the cavity enclosed by the overarching secondary wall is known as the pit chamber and the opening in the border, the pit aperture (Fig. 4.4). In the simple pit, there is no such overarching. Pits help in the transport of substances from one cell to another.

A pit in a wall of cell is usually situated opposite a complementary pit in the wall of the adjacent cell. This combination of two pits is called *pit pair* (Figs. 4.3 and 4.5). The pit membrane is common to the pit pair. It is constituted by two primary walls and the middle lamella. In the bordered pits of conifers, the pit membrane has a thickening called torus. The part of the membrane surrounding the torus, called margo, is flexible. It has radially arranged microfibrils which are embedded in the surrounding primary wall. They hold the torus in position. There are small openings between the fibrils of the margo. Aqueous substances pass from one cell to another through these openings. Bordered pits occur in tracheids of pteridophytes, gymnosperms and in the secondary xylem of dicotyledons.

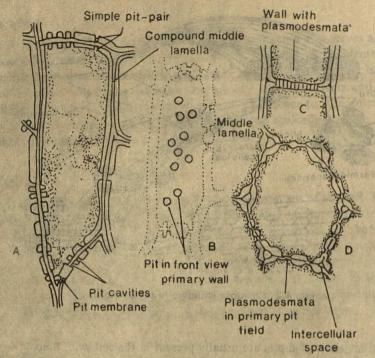


Fig. 4.3 A—Sectional view of ray cell showing simple pits and pit pairs. B—Same, showing pits in front view. C & D—Parenchyma cell without secondary wall. C—Plasmodesmata dispersed throughout the wall. D—Plasmodesmata restricted to primary pit.

Three - layered secondary wall

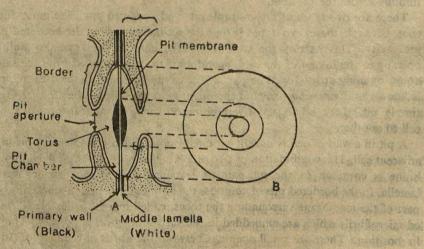


Fig. 4.4 Sclereid cells with secondary walls and simple pits.

A—Sectional view, B—Face view.

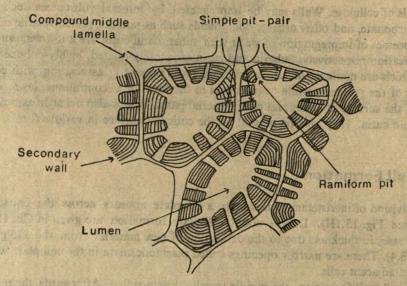


Fig. 4.5 Diagram of bordered pit pairs.

#### Plasmodesmata

These are protoplasmic connections between adjacent cells. They pass through the pores situated in the primary pit fields (Figs. 4.3C and D). The presence of tubules in the plasmodesmata has been reported. These tubules are in continuity with the endoplasmic reticulum. Plasmodesmata are concerned with the transport of materials. They are said to be also involved in the conduction of stimuli.

# Chemical Composition

The middle lamella consists chiefly of pectic substances which are derivatives of polyglacturonic acid. They occur in three common types, namely, protopectin, pectin, and pectic acid and are, as previously stated, amorphous colloidal substances. Being highly hydrophilic and plastic, it is likely that they help in maintaining a state of high hydration in young cell walls.

Cellulose is the most common compound in plant cell walls. In fungi, the cell wall consists of chitin. Besides cellulose, hemicellulose and pectin occur in the primary cell wall while in the case of the secondary cell wall, lignin is present in addition to cellulose and hemicellulose. Lignin is an organic compound of high carbon content which is distinct from carbohydrates. It consists of aromic groups related to coniferyl alcohol, with side chains. It may occur in the middle lamella, the primary wall or the secondary wall. Its presence makes the cell wall rigid and strong.

The process of lignification starts usually in the middle lamella and then extends centripetally through the primary and secondary walls. It is deposited in the spaces between the microfibrils of cellulose. Walls may be impregnated by mineral substances such as silica, calcium carbonate, and other organic compounds such as tannins, resins, volatile oils, etc.

The processes of impregnation of walls with suberin and cutin are known as suberization and cutinization respectively. Suberin and cutin are closely-related, highly-polymerized compounds which are made up of fatty acids. Suberin is present in association with cellulose in cork cells of the periderm while cutin occurs in the form of a continuous layer called the cuticle on the surface of epidermal cells of aerial parts. Waxes also occur in association with sugerin and cutin. They may be present on the cuticular surface in various forms.

#### **Cell-Wall Formation**

During division of meristematic plant cells, a cell plate appears across the equator of the spindle (see Fig. 13.1H). Details about cell-plate formation are given in Ch. 12. The cell plate increases in thickness due to the deposition of new material from the Golgi complex (see Fig. 8.4). There are narrow openings called plasmodesmata in the cell plate which serve to connect adjacent cells.

During cell-wall formation, the middle lamella develops first. Afterwards, the primary wall is laid down along the middle lamella. The secondary wall is formed between the primary wall and the cytoplasm of the cell, and consists of three layers of parallel fibres.

# SUMMARY

- 1. Plant cells possess non-protoplasmic walls which are complex in nature.
- 2. The cell wall comprises three layers: the middle lamella, the primary wall and the secondary wall. The last-named layer is laid down on the primary wall after the cell ceases to enlarge. The main constituents of the secondary wall are cellulose, hemicellulose and lignin. Their main function is mechanical.
- 3. The matrix of the cell wall is composed of polysaccharides. There is a network of microfibrils therein. There are simple pits and border pits. The adjacent cells are in communication with each other through protoplasmic connections called plasmodesmata which pass through the pores situated in the primary pit fields.
  - 4. The middle lamella consists mainly of pectic substances which occur in three types: protopectin, pectin and pectic acid. Cellulose is the most common compound in cell walls. The process of lignification usually begins in the middle lamella and then extends centripetally through the primary and secondary walls. The processes of impregnation of walls with suberin and cutin are known as suberization and cuticularization respectively.
- 5. During cell division, a cell plate is formed across the equator of the spincle. The middle lamella develops first and the primary wall is laid down along it. The secondary wall is formed between the primary wall and the cytoplasm of the cell.

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Muhlethaler, K. (1967), "Ultrastructure and formation of plant cell walls", Ann. Rev. Plant. Physiol: 18: 1.

# 5. Cytoplasm

If living cells, including bacterial cells, are analysed chemically, it will be seen that they are similar to a large extent. The basic molecular fabric of a primitive cell consists of ribosomes, RNA molecules, globular and fibrous proteins (which include many enzymes), small molecules and water. Such a fabric is also found in the cytoplasmic matrix or substratum of higher cells.

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While the cell was being evolved, many intercellular membranes were formed. Consequently, DNA and other molecules were fixed in the nucleus. Other cell organelles such as mitochondria, chloroplasts, and centrioles became a part of the cytoplasm. In certain embryonic plant and animal cells, most of the cytoplasm comprises the matrix and ribosomes while the intercellular membranes are little developed.

The cytoplasmic matrix is the most important part of the cell. It carries out the biosynthetic functions of the cell and contains the enzymes necessary for the production of energy primarily by a process of anaerobic glycolysis.

The collodial properties of a cell like viscosity changes, intercellular motion (cyclosis), amoeboid movement, spindle formation and cleavage of cell, are dependent mostly on the cytoplasmic matrix. Moreover, the matrix of the cytoplasm is the seat of many fibrillar differentiations found in specialised cells such as keratin fibres, myofibrils, microtubules and filaments.

The term groundplasm is usually used in connection with the matrix. It indicates the homogeneous plasma remaining after the removal of all organelles that can be detected with the aid of electron microscopy. If the fixation is perfect, the groundplasm appears to be of a very fine granular structure. The granules have diameters varying from over 100 Å down to the limits of the resolving power of an electron microscope.

In classical cytology, a distinction is made between peripheral homogeneous ectoplasm or hyaloplasm (term used by Pfeffer in 1890) and endoplasm or granular plasma within a cell (Pringsheim, 1854; Hofmeister, 1867). Under a light microscope, groundplasm appears as hyaloplasm. It is the capacity of transforming sol-gel in the reverse direction (Fig. 5.1). In the liquid state, it is a 'gel' solution and shows the property of elasticity. The laws of hydrostatics are not applicable to sol-gel reactions of colloids. Cytoplasmic gel does not solidify under high hydrostatic pressure (e.g. 300 atm.) but on the contrary it becomes reversibly liquid. It is because of the granular structure of the cytoplasmic matrix that the special properties such as flow elasticity and volume increase on setting to a gel are acquired.

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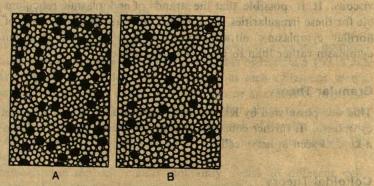


Fig. 5.1 Transformation of sol into gel A—Sol. B—Gel. a reache with a gain cose & and a manufacture to replicate account the description of the alle

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## Ultrastructure of Cytoplasm

As regards the ultrastructure of cytoplasm, various theories were proposed and they form a part of classical cytology. Although they are not valid anymore, it is interesting to know them so that we can learn how structures have played a part in the experiments carried out during that period. Moreover, it should be borne in mind that since the plasma system is extremely heterogeneous, it is extremely difficult to have conclusive data.

## Berthold's Emulsion Theory (1886)

According to this theory, droplets are embedded in a semiliquid phase. This was based on the observation of mitochondria, plastids and other particles under a light microscope. Nothing was known, however, about their nature.

# Butschli's Form or Alveolar Theory (1892)

This theory was discarded because it was based on artifact due to fixation (Frey-Wyssling and Muhlethaler, 1965). Butschli regarded cytoplasm as a kind of foamy emulsion of two substances intimately mixed. One of them was supposed to be the ground substance of a colloidal nature and the other spheres of more liquid material suspended in it. He considered the cytoplasm as comprising a large number of alveoli or minute bubbles in a comparatively viscid medium.

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# Flemming's Fibrillar Theory (1879)

This theory supposes the occurrence of filamentous elements in the cytoplasm. This was based on observations made in vivo. It lays stress on the fact that heavy particles wandering through the plasma under the influence of the gravitational field, are continuously obstructed by invisible threads and thus, cytoplasm cannot be homogeneous. It is, therefore, thought to be full of invisible structures possessing a higher density and not uniformly

#### 42 Cytology and Genetics

viscous. It is possible that the strands of endoplasmic reticulum may be actually responsible for these irregularities. Hence, the observations on the basis of which the theory claims fibrillar cytoplasmic ultrastructure appear to be due to the organelles embedded in the cytoplasm rather than to the groundplasm itself.

#### **Granular Theory**

This was postulated by Klein, Cornoy and others. It lays stress on the fibrous make-up of cytoplasm. It further conceives that the fibres are knotted into an intimate network of such a kind as seen in nerve cells.

# **Colloidal Theory**

This theory, put forward by Fischer in 1894, suggests that cytoplasm (protoplasm) is colloidal in nature. Hardy had made a similar suggestion in 1899. According to this theory, cytoplasm has a higher water content than other constituents of the cell and its particles are of varying sizes. These particles may be soluble in water forming a homogeneous mass. If they are insoluble, the mass is heterogeneous. The suspension of particles in the cytoplasm indicates its colloidal nature. The various components give four kinds of appearances to cytoplasm, as contemplated by the above-mentioned theories.

# Ergastoplasmish affections a safet for the Pills construct to the same of determination of

At the end of the eighteenth century, it was discovered that portions of the ground cytoplasm in certain cells had a differential staining property. Since these regions stained with basic dyes, just as the nucleus did, they were termed basophilic or chromodial cytoplasm. Garnur, however, preferred the term *ergastoplasm*, thus implying biosynthesis as its fundamental role. Ergastoplasm includes the basophilic regions of the ground cytoplasm, for example, Nissl bodies of nerve cells.

Butschli's Form or Aiveolar Theory (1882)

ostances in time leiv mixed. One of them was supposed to be

# Early Submicroscopic Studies of Cytoplasm

Even before the invention of the electron microscope, cytologists had some idea about the finer organisation of the hyaloplasm. In 1910, Gaidakow using an ultramicroscope found the parts of the cytoplasm containing refractive bodies. Bayliss (1924) also examined hyaloplasm of amoebae with the ultramicroscope and observed small particles undergoing Brownian movement. Studies with a polarisation microscope put forth additional data. Hyaloplasm was found to be isotropic and appearing dark between Nicol prisms. In some regions of the cytoplasm, a weak birefringence was, however, detected. Experiments carried out on cultured cells showed the existence of asymmetric particles in the cytoplasmic matrix.

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Mechanical Properties

## Ultrastructure of Cytoplasmic Matrix

Some racchanical properties such as control little clasticity, cohesion, Tigility and move-If the cytoplasmic matrix is examined under an electron microscope, it appears homogeneous or finely granular. Its electron density is low. Fine filaments of less than 100 Å can be seen easily when their arrangement is parallel. It is presumed that such a filament would polymerize out of the matrix in response to environmental stress or as a part of organisation necessary for the division of cells. A further assumption is that these filaments give the cell its gelled consistency. The construction of the framework of keratin fibrils and myofibrils depends on the organisation of filaments of similar size and their relation to other cytoplasmic components.

The macromolecular organisation of the cell matrix not only varies in different cells but also in different regions of the matrix. Filaments are connected on both sides of the attachment of desmosomes. These facts indicate that the components are elongate and by means of which the structural relationship of protoplasm is maintained. It is assumed that in the network formed by these structural proteins, polypeptide chains are probably held together by cross-linkages of hydrogen bonds or van der Waal's forces or by stronger valences. Changes caused in the strength of these cross-linkages, degree of folding, or in the length of aggregation of chains may convert a sol into a gel in a particular region of protoplasm. It is not necessary that these filaments should always remain united since there may be long-range forces holding them together.

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#### Physicochemical Properties of Cytoplasmic Matrix Challeton Crounisation of Cytopies no Natrix.

# Egg Polarity

If certain eggs are centrifuged, components of the cell become stratified but the original polarity acquired during the cleavage of the cell is maintained. This fact suggests that polarity is determined by the cell matrix and centrifugation cannot change it.

# requiring ATF are detected in the soluble fraction as also (WA. Thus, 20 cloments have **Hydrostatic Pressure Action**

If moderate hydrostatic pressure (e.g. 5000 1b/sq inch) is applied, it inhibits certain physiological activities with the result that these activities are reduced to solation-gelation changes in the cytoplasm, e.g. cyclosis, amoeboid movement, cell division, etc. The inhibition takes place owing to the degree of solation caused by the pressure induced in the plasma gel system and to the resulting changes in viscosity. With the application of pressure, particularly when the cytoplasm is packed with particles, there is a change in the cytoplasmic activity, which results in an increase in viscosity.

## Change in Viscosity College to Cartering the College to Cartering the College to Cartering the Carte

The viscosity of different cells can be changed by environmental or internal factors. Hypertonic solutions increase viscosity while hypotonic ones decrease it. Continuous changes take place in the amoeboid movement and during the mitotic cycle.

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#### **Mechanical Properties**

Some mechanical properties such as contractility, elasticity, cohesion, rigidity and movements within the cell are related to the cytoplasmic matrix and even to the components such as the vacuolar system. Although the membrane system is relatively dense, the matrix is the least compact part of the cytoplasm.

#### pH of Cytoplasm

The cytoplasmic matrix is generally slightly acidic having a pH of about 6.8. The vacuolar content may either be basic or acidic. In the latter case, the pH may be as low as 5.0. The pH is about 7.6 to 7.8 in the case of aqueous nucleoplasmic matrix. The characteristic property of protoplasm is its power of buffering. The pH of the cell can be changed by addition of acids or alkalies to the medium or by injecting them into the cell. However, the original pH is rapidly restored if the cell vitality has not been changed.

#### **Osmosis and Osmatic Pressure**

One of the most significant physical phenomena connected with colloidal membranes is osmosis and osmotic pressure, which may be very strong. Osmosis and osmotic pressure have a very important role in the life process. Many important processes in cells depend upon the semipermeability of their membranes. Owing to this property, the cells remain turgid and so become rounded on outside. In such a case, the osmotic pressure inside and outside of the plasma membrane is equal.

# Chemical Organisation of Cytoplasmic Matrix

The protein content of the cytoplasmic matrix is mostly globular proteins. However, fibrillar structures may be formed in certain physiological processes. Soluble proteins and enzymes constitute 20 to 25% of the total protein content of a cell. Of the important soluble enzymes found in the matrix are those taking part in glycolysis and activation of amino acids which subsequently leads to protein synthesis. The enzymes of many reactions requiring ATP are detected in the soluble fraction, as also tRNA. Thus, 20 elements have been demonstrated necessary for life.

# **Chemical Components of Cytoplasm**

About 14 elements have been actually detected in protoplasm and 12 of them are universally present. These are: C, H, O, N, S, P, Cl, Na, K, Ca, Mg, Fe, and I. It is interesting to note that 96% of the entire body of an organism consists of only O, C, H and N in their various combinations. The 20 essential elements are classified into three principal categories: (i) major constituents, (ii) trace elements, and (iii) ultratrace elements.

Major constituents
 Hydrogen

Function

Elements required for formation of organic

compounds of the cell

Carbon Nitrogen Oxygen

S TO Y

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phase form ALT, which is their lation. An important group is n

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| 2. Trace elements | 2. | Tr | ace | ei | lem | ent | S |
|-------------------|----|----|-----|----|-----|-----|---|
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| Connected with internal strength. Chiefly in   |  |
|--|--|
| liquid outside cells                           |  |
| Cofactor of many enzymes                       |  |
| Constituen: of nucleic acids, connected with   |  |
| energy-transfer reactions                      |  |
| One of the constituents of proteins and other  |  |
| important compounds                            |  |
| One of the principal anions                    |  |
| Universally found in cell, important in muscle |  |
| contraction, nerve stimulation, etc.           |  |
| Cofactor of enzymes, important constituent     |  |
| of membranes and their controller              |  |
|  |  |

#### 3. Ultratrace elements

| Boron              | Important in plants, usually cofactor of enzymes |
|--------------------|--|
| Silicon            | Occurs abundantly in lower organisms             |
| Vandium            | Occurs in some chromoplasts of lower organisms   |
| Manganese          | Cofactor of many enzymes                         |
| Iron               | Cofactor of many oxidative enzymes               |
| Cobalt 100 off 020 | Constituent of B <sub>12</sub> vitamin           |
| Copper voto neo fi | Cofactor of many oxidative enzymes               |
| Zinc               | Cofactor of many enzymes                         |
| Molyhdenum         | Cofactor of a few enzymes                        |

The chemical components of a cell are classified into inorganic and organic materials. Water and mineral ions come under the former while proteins, carbohydrates, nucleic acids, lipids, etc. under the latter.

The organic components constitute more than 35% of the protoplasm and most of them are proteins, carbohydrates, lipids and enzymes. Along with these compounds water, gases and certain inorganic salts are also present.

The relative percentages of the principal organic and inorganic compounds usually found in active protoplasm are as follows:

| of Chemical energy from | (per cent) |
|-------------------------|------------|
| Water                   | 75-85      |
| Protein                 | 10-20      |
| DNA TO TO TO THE WOOL   | 0.4        |
| RNA ablacti vosta       | 0.7        |
| Lipid man old ball      | 2-3        |
| Carbohydrates           | 1          |
| Other organic matter    | 0.4        |
| Inorganic materials     | 1.5        |

#### Free and Bound Water

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Water is the most abundant component of cells with a few exceptions like bones and enamel. It is a natural solvent for mineral ions and other solvents. It also serves as a dispersion medium of the colloidal system of protoplasm. Metabolic activity cannot take place without water since physiological processes occur only in an aqueous medium. Molecules of water take part in many enzymatic reactions going on in cells. They can also be formed as a consequence of metabolic processes.

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Water is found in cells in the free as well-bound states. Free water constitutes 95% of the total cellular water. It acts chiefly as a solvent for solutes and as a dispersion medium for the colloid system of protoplasm. Bound water is only 4-5% of the total cellular water. It is loosely bound to the proteins by hydrogen bonds and other forces. It includes water which is not mobilised and contained within the fibrous structure of macromolecules. A water molecule acts as a dipole because of the distribution of asymmetric charges. This is illustrated as follows:

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On account of this polarity, water is capable of binding electrostatically to both positively and negatively charged groups in the proteins. Hence, each amino group in a protein molecule can bind 2 to 6 molecules of water.

Water is used for the elimination of certain substances from the cell as well as for absorbing heat, since it has a high coefficient of specific heat which can prevent drastic changes in temperature inside the cell.

The water content of an organism is related to its age and metabolic activity. It is the highest (90-95%) in the embryo while it decreases progressively with age. It also varies in different tissues, its percentage being dependent on metabolism.

#### Salt and lons

Salts dissociate into anions (e.g. Cl) and cations (Na and K). These are important for the maintenance of the acid-base equilibrium of the cell and osmotic pressure. When the ions are retained, the osmotic pressure increases and water enters the cell. Inorganic ions like Mg<sup>++</sup> are essential as cofactors in the enzymatic activity while those like inorganic phosphate form ATP, which is the main source of chemical energy through oxidative phosphorylation. An important group is phosphate. It is associated with phosphoprotein, phospholipid and nucleotide. The reactions of different ions with each other is a kind of regulative action. It results in neutralisation of toxic effects of any given ion.

In the organisation of organic compounds like amino acids, sulphur is an important constituent. Inorganic ions like Mn<sup>++</sup> and Mg<sup>++</sup> may act as cofactors in the case of action of specific enzymes.

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# **Proteins and Amino Acids**

Proteins form the framework of protoplasm. Because of them, the latter has become complex and acquired specific properties. They constitute enzymes and the contractile machinery of the cell, and contain C, H, O, N, S, and P. These usually occur in the form of an ingredient of the protoplasmic gel, and are classified into (i) simple, (ii) conjugated, and (iii) derived proteins. Simple proteins are primarily of four kinds: (i) albumin, (ii) globulin, (iii) histone, and (iv) protamine.

#### Simple Proteins

- 1. Albumin Soluble in water, coagulated by heat
- 2. Globulin Insoluble in water, soluble in alkaline acids and salt solutions
- Soluble in water, insoluble in diluted ammonia with strong alkaline reaction. Some combine with nucleic acid to form nucleohistones. They are less basic and formed in many cell nuclei.
- 4. Protamine Soluble in water, incoagulable by heat with strong alkaline reaction. When combined with nucleic acids, nucleioprotamines are produced.

#### Conjugated Proteins

When simple proteins are combined with other substances of an organic nature belonging to the prosthetic group, conjugated proteins are formed. When these are hydrolysed, alpha amino acids and an organic compound are produced. The important conjugated proteins are nucleoproteins, glycoproteins, lipoproteins, chromoproteins comprising haemoglobin, haemocyanin and respiratory enzymes like cytochromes, flavoproteins, etc.

#### **Derived Proteins**

These are denatured (coagulated) and partly hydrolysed proteins. Proteases, peptones and polypeptide come under this category.

Proteins are composed of amino acids. The latter are derived from aliphatic acids (e.g. acetic acid) by the substitution of an amino group (NH<sub>2</sub>) instead of a hydrogen group.

$$RCOOH + RNH_2 \rightarrow R - CO - NH - R' + H_2O$$

The linkage—CO—NH—is known as a peptide bond. A chain formed by the combination of two amino acids is known as a dipeptide. If the number of amino acids are few, the chain is termed oligopeptide (Fig. 5.3A) and if it involves many amino acids, it is termed polypeptide (Fig. 5.2). The arrangement of amino acids in the protein chain is not always linear and it may contain one or more turns or folds (Fig. 5.2). The polypeptide chain made of amino acids is known as the primary structure of the protein molecule. It is the most important

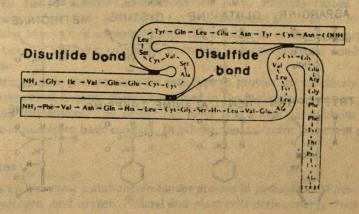


Fig. 5.2 Primary structure of insulin

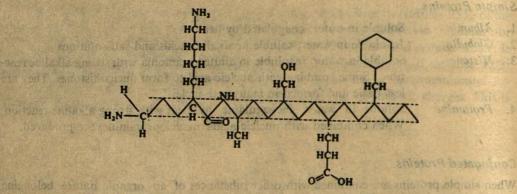


Fig. 5.3A Polypeptide chain formed by glycine, lysine, alanine, serine, glutamic acid and phenyl alanine.

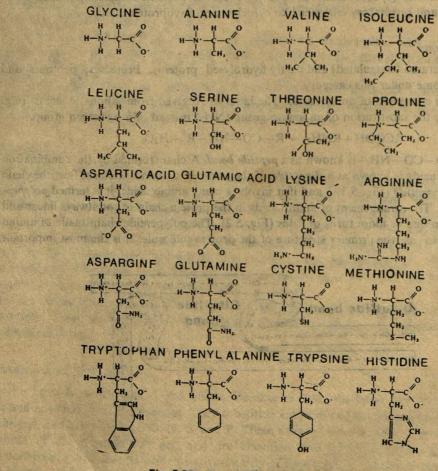


Fig. 5.3B General 20 amino acids in proteins.

and specific structure. It determines the secondary and tertiary structures to a certain extent. Aggregation of the protein units containing the secondary and tertiary structures results in a quaternary structure. There are 20 amino acids (Fig. 5.3B) and many more proteins which are biochemically important.

Proteins are usually large molecules with a high molecular weight ranging approximately between 12,000 and over 2,000,000. Several proteins act as enzymes. Ribonuclease is the enzyme whose structure was first determined. It consists of several turns in a chain of 19 different amino acids. Its molecular weight is 14,000. Some enzymes require addition of a non-protein for their activity. If this addition is of an inorganic nature, it is called a prosthetic group. If the nature is inorganic, the term cofactor is used. A cell contains several enzymes. They are associated with several cellular components such as the endoplasmic reticulum, mitochondria, Golgi complex, plastids, etc.

Proteins are amphoteric compounds since they contain both acid (COOH) and base (NH2) groups. The acidic groups lose a proton and become negatively charged. Such a type occurs in diacidic amino acids such as aspartic and glutamic acid. Here dissociation of free COOH radicle takes place as follows:

The basic groups gain a proton and thus become positively charged, for example, lysine and arginine which possess two basic groups each. All these inorganic groups together with the terminal-free COOH and NH2 groups give rise to acid-base reactions of proteins and because of them, protein molecules acquire electrical properties.

The molecular weight of insulin is 36,500 while that of nucleohistone is 2,300,000. Differences found in proteins of different species in tissues or cells depend in part on amino acid residues.

The dissociation of different acidic and basic groups takes place at different H+ concentrations of the medium. Therefore, it influences the total charge of the molecule to a large extent. In case of all proteins, there is a specific pH at which the net positive and negative charges are zero. The pH is known as the isoelectric point. Proteins placed in an electric field at the isoelectric point, do not migrate to the cathode and anode at the lower and higher pH's respectively. Many physiochemical properties of proteins at the isoelectric point show unique properties, e.g. viscosity, solubility, hydration, osmotic pressure, conductivity, minimum value, etc.

The isoelectric point is characteristic for each type of protein. Its specificity depends upon the type and amount of isogenic groups contained in the molecule. For example, in the case of histones and protamines, the isoelectric point is at a high pH of about 10-12 because of the presence of numerous basic residues. In gelatin, the isoelectric point is at pH 4.7.

#### Carbohydrates

Carbohydrates are the most common substances present in protoplasm. These are composed of carbon, hydrogen and oxygen. Animal and plant cells derive energy from them. Carbohydrates are the important constituents of cell walls in many plant cells and serve as

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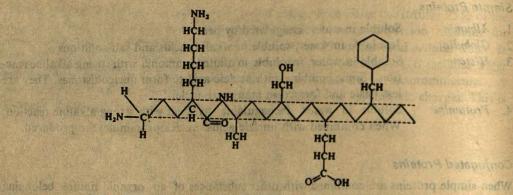


Fig. 5.3A Polypeptide chain formed by glycine, lysine, alanine, serine, glutamic acid and phenyl alanine.

Fig. 5.3B General 20 amino acids in proteins.

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supporting elements. Green plants synthesize various kinds of carbohydrates directly from CO<sub>2</sub> and H<sub>2</sub>O in the presence of light. A few carbohydrates are present in animal tissues, some important ones being glucose, glactose, glycogen, amino sugars and their polymers.

Biologically important carbohydrates are divided into three kinds: (i) monosaccharides, (ii) disaccharides, and (iii) polysaccharides. The first two form sugars. They are readily soluble in water and penetrate easily through dialyzing membranes. They can be crystallised while polysaccharides do not crystallize. The latter also do not pass through membranes.

#### Monosaccharides

These are simple sugars. Their empirical formula is  $C_n(H_2O)_n$ . According to the number of carbon atoms present in the molecule, they are classified as trioses, pentoses, hexoses and heptoses. Of these, the biologically important sugars in the cell are pentose and hexose. Ribose and deoxyribose sugars are pentose sugars and they form components of molecules of nucleic acids. Hexoses are important for food and nutrition. Of them, glucose and fructose are important. The former plays an important role in the metabolism. All carbohydrates which are taken as food are ultimately broken into glucose in the body. Although fructose has a composition similar to glucose, it differs in the chemical properties and physical role. This is because of the differences in the position of the —OH group in the two molecules, which are shown below:

#### Disaccharides

These are sugars formed by the condensation of two molecules of monosaccharides with the loss of one molecule of water. Its empirical formula is  $C_{12}H_{22}O_{11}$ . The chief sugars of this group are sucrose and maltose in plants and lactose in animals. All these sugars are derived from the condensation of hexoses.

#### Polysaccharides

These are formed by the condensation of many molecules of monosaccharides with a corresponding loss of water molecules. Their empirical formula is  $(C_{16}H_{10}O_5)^n$ . When hydrolysed, they yield molecules of simple sugars. Starch, glycogen and cellulose are biologically important polysaccharides. The first two are the reserve substances in plant and animal cells

on Genetics

respectively, while cellulose is the characteristic structural component of plant cells. Starch (C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>)<sup>n</sup> is a mixture of two long polymer molecules—linear amylose and branched amylopectin. It has no structural importance in animals. Glycogen (C6H10O5)m is an animal starch. It is a polymer composed of many molecules of glucose and therefore constitutes an important reserve material of energy in the body. Cellulose is composed of hundreds of monosaccharides. It has a significant role in the formation of cell walls and a series of other structures which form the supporting skeleton in plants.

#### Lipids

The characteristics of this large group of compounds are their relative insolubility in water and solubility in organic solvents. The predominance of long aliphatic hydrocarbon chains or of benzene rings is responsible for this general property of lipids and related compounds. These structures are non-polar and hydrophobic. In many lipids, these chains may be attached at one end to a polar group, so as to make it hydrophilic and capable of binding water by hydrogen bonds. Lipids are classified into the following:

# during interplane the first are a remained with acceptance to foun ancien-1. Simple Lipids

These are alcoholic esters of fatty acids and include the following:

- (i) Natural fats or glycerides (triglycerides), which are the triesters of fatty acid and glycerol. They are further divided into fats and oils. Examples of some common fats are lard, tallow etc., while castor oil, fish oil, olive oil, etc. are examples of some common oils.
- (ii) Waxes, which are the esters of fatty acids with alcohol other than glycerol, e.g. bee's These dougles of senters are at (45% of 1874) the sentent of the s

# in to reposed to these a se bornet saide deverting and the said stick produced bear 2. Steroids atom and man the their modern steady of a ministration seems of section of their

These are the complex lipids. Bile acid, sex and adrenal hormones, vitamin D, etc. are some examples of steroids. Steroids with an OH group are known as sterols, e.g. cholesterol, which is the principal constituent of wool fat. It is also present in bile, brain, adrenal glands and other organs. is rulk'd that waterwall. Whelevid a six more than ested as a mere sides Prospecte act

# 3. Conjugated Lipids form evisions are to decrease add garately separately and account of

to discharge one military will show a self-based managed On hydrolysis, these compounds yield other compounds in addition to alcohol and acids. These include compounds such as glycolipids and phospholipids. Trade in the standard with the standard of the tender of the tenders of the purple become at

and the state of the state and state of the state of the

#### 4. Carotenoids

These are plant and animal pigments. Chemically, they are hydrocarbons with the general formula C<sub>40</sub>H<sub>36</sub>. The orange-yellow colour of carrots is because of the presence of carotene. In the animal body, carotenes are frequently found in the skin cells. It is owing to their presence that the skin assumes a deep colour. They have a wide distribution in the plant kingdom and occur in three forms, viz. α-, β- and γ-carotenes. Xanthophill is, however, masked by chlorophyll. When chlorophyll diminishes in autumn, the presence of lutein is revealed.

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Other biologically important pigments are lactoflavin in milk and riboflavin (vitamin B<sub>2</sub>). These flavoproteins form important enzymes.

In organisms the role of lipids varies to a large extent owing to their location and distribution. Glycerides are used as stores of energy. Phospholipids and cerebrosides occur chiefly in the nervous tissue as constituents of myelin. Among steroids, bile acids emulsify proteins. This emulsification helps in the process of digestion. In some animals such as seals and whales, glycerides protect against cold and injury.

#### **Nucleic Acids**

These are of the utmost biological importance. They occur in the form of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Nucleic acids are found in all living organisms. Some viruses such as tobacco mosaic virus (TMV) and poliomyelitis virus have only RNA while in others, e.g. bacteriophages, vaccinia and aldenoviruses, only DNA is present. In bacteria and higher cells, both the nucleic acids occur. DNA is chiefly present in the nucleus and forms a part of chromosomes at the time of cell division. It is in the chromatin during interphase. In the nucleus, it combines with proteins to form nucleo-proteins. It is also present in chloroplasts, mitochondria and probably in other self-replicating organoids. RNA is found in the nucleus as well as in the cytoplasm. In the nucleus, it occurs in the nucleolus, nucleoplasm and chromatin. Ribosomes are present in the cytoplasm, with RNA constituting the major portion.

#### Chemical Structure of Nucleic Acids

These consist of pentose sugar (ribose in RNA and deoxyribose in DNA), nitrogen bases and phosphoric acid. They are long polynucleotides formed as a result of linkage of many nucleotides. A sugar molecule, a phosphate molecule and a nitrogen base molecule together form a nucleotide (Figs. 5.4 and 5.5).

Each monomer of the nucleic acid is a nucleotide which is a combination of one molecule of phosphoric acid, one of pentose sugar and the other of purine or pyrimidine nitrogen base. The part of the nucleotide formed because of the condensation of a base and a sugar is called the *nucleoside*. Nucleotides are phosphoric esters of nucleosides. Phosphoric acid links the nucleotides by joining the pentose of two consecutive nucleosides with an esterphosphate bond. These bonds link carbon 3' in one nucleotide with carbon 5' in the next nucleotide.

There are two kinds of pentose sugars—ribose in RNA and deoxyribose in DNA. The Feulgen reaction, which is specific for DNA, is due to deoxyribose. The purine bases are mainly adenine and guanine and are found in both the nucleic acids. Pyrimidine bases are mainly cytosine, thymine and uracil. Whereas cytosine is present in both DNA and RNA, thymine is found only in DNA and uracil in RNA. 5-methylcytosine and 5-hydroxymethylcytosine may occur in DNA of bacteriophages in extremely small amounts. All nitrogen bases have double bonds between the carbons alternating with single bonds.

It is possible to examine the characteristics of some natural substances and synthetic dyes like acriflavin, acridine, orange, quinacrin mustard with the help of fluorescence microscopy. These dyes have the property of combining with DNA or RNA strands. They have a

specific fluorescence colour and hence, it is possible to detect the presence of DNA or RNA by using these stains.

Fig. 5.4 Chemical structure of RNA polynucleotide chain.

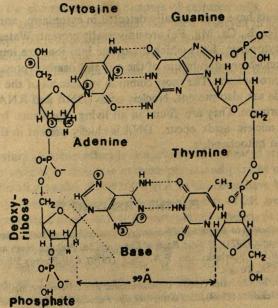


Fig. 5.5 Part of DNA molecule showing bonds between two complementary parts of bases (cytosine-guanine; adenine-thymine).

Table 5.1 Summary of chemical components, localisation and properties of DNA and RNA

|   | DNA  | RNA  |
|---|--|--|
| Localisation Pyrimidine bases Purine bases Pentose sugar Chemical reaction Histological stain | Nucleus, mitochondria and chloroplasts Cytosine, thymine Adenine, guanine Deoxyribose Diphenyl amine Feulgen | Cytoplasm; nucleolus and chromosomes Cytosine, uracil Adenine, guanine Ribose Orcinol Acidophilic stain, after reaction of ribonuclease enzyme |

#### SUMMARY

- 1. The cytoplasmic matrix is the most important part of the cell. It carries out biosynthetic functions of the cell. It contains necessary enzymes for the production of energy, primarily by a process of anaerobic glycolysis. Various colloidal properties of the cell are dependent mostly on the cytoplasmic matrix which is also the seat of many fibrillar differentiations.
- 2. As regards the ultrastructure of cytoplasm, various theories have been proposed. Under an electron microscope the cytoplasmic matrix appears homogeneous or finely granular. Its electron density is low. Fine filaments less than 100 Å are seen when their arrangement is parallel. Its pH is about 6.8. It exhibits hydrostatic pressure action and changes in viscosity. It possesses mechanical properties like contractility, elasticity, cohesion and rigidity.
- 3. About 14 elements have been actually detected in cytoplasm, and 12 of them (C, H, O, N, S, P, Cl, Na, K, Ca, Mg, Fe) are universally present. Water is the most abundant component of cytoplasm. Salts which dissociate into ions are important in the maintenance of the acid-base equilibrium of the cell and osmotic pressure. Proteins form the framework of protoplasm. The most common substances of the cytoplasm are carbohydrates. Lipids are also present. Nucleic acids (DNA and RNA) are of the utmost biological importance. They are found in all living organisms. In bacteria and higher cells both the nucleic acids occur. DNA is chiefly present in the nucleus and also in chloroplasts and mitochondria.

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# 6. Endoplasmic Reticulum

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# **Historical Background**

In 1945, Porter, Claude and Fullam observed a reticulum or network of strands in the cytoplasm of thinly-spread tissue culture cells. The reticulum was found to be associated with vesicle-like bodies. Further studies in 1947 revealed that the vesicular bodies were, in fact, interconnected to form a complex network. In majority of the cases, this network was confined to the endoplasmic or inner part of the cytoplasm, and therefore it was termed the endoplasmic reticulum by Porter and Kallman (1953). After about five years, the examination of thin sections of cells using an electron microscope confirmed the observations of Porter et al. The form and distribution of the endoplasmic reticulum in living tissue culture cells were studied by Fawcett and Ito (1958) and Rose and Pomerat (1960) with the aid of a phase-contrast microscope. Even the endoplasmic reticular elements in their characteristic form were observed in the cinematographic pictures of cultured cells.

So far, the presence of endoplasmic reticulum has been observed in all types of cells that have been studied except red blood corpuscles. It has, however, often been observed in eggs and embryonic or undifferentiated cells. A few vesicles have been observed in spermatocytes also. Thus, the development of the endoplasmic reticulum varies in different cell types.

### Structure

The endoplasmic reticulum (ER hereafter) is constituted of elongated strands or trabiculae. The strands form large vesicles or cisternae at some places and parallel membranes at others (Figs. 6.1 and 6.2). Small granules about 100-150 Å in diameter have been observed in the narrow space between these two elements. Since some elements of ER are studded with small granules (Fig. 6.3), it presents a rough surface (ergastoplasm) whereas at other places because of the absence of these granules, it appears smooth. Thus, morphologically there are two basic types of ER: (i) rough or granular type and (ii) smooth or agranular type. The latter type is less stable than the former. It vesiculates if there is even a slight damage or death of the cell. The granules are about 150 Å and osmiophilic. They are rich in RNA and their number is approximately proportional to the RNA content of ground substance. Now it is known that they are ribsomes and take part in protein synthesis.

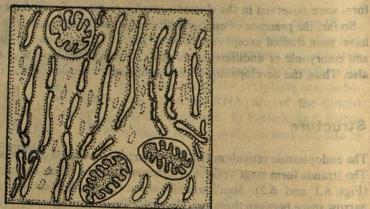
There is great variation in the amount of ER in a cell with the age and function of the

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cell and extra-conditions. Its strands consist of double membranes and the space between them varies. When the strands widen out into vesicles, often the vesicles form chains and are connected with one another by canalicules or larger ventricles. In this way, caverns are



Fig. 6.1 Part of electron-micrograph (diagrammatic) of pancreatic exocrine cell of guinea pig. At the bottom, nuclear boundary; on the upper side, two mitochondria; and on the right side, one are seen. In the middle, there is ER with tubules with rows mand the first culture covered with RNP granules. (With permission of Dr. K. M. Mann, Editor, Scope Publications-Scope Monograph on Cytology.



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Fig. 6.2 Part of the diagram of the electron-micrograph of liver cell cytoplasm. Rough vertical rows of lamellae are seen. They are covered with electron-dense granules (Palade's small granules in ribosomes). Note that they are also distributed in cytoplasm and to non-sult bank and supply in-between lamellae. (Redrawn from Wilson and Morrison, Cytology, 1966).

formed in the groundplasm. Cisternae or lamellae are long and flattened units with a thickness of 40 to 53 m $\mu$ . They are frequently arranged in parallel stacks. Generally, vesicles are round-shaped and their diameter varies from 25 to 500 m $\mu$ . Tubules are about 50 to 100 m $\mu$  in diameter and appear circular in electron micrograph sections. They are generally more diverse and usually found in secretory cells. The presence of vesicles, cisternae or tubules clearly indicates that there is a hollow or capillary system in the cytoplasm interlacing the entire cell. These hollow spaces are connected to each other and covered by a unit membrane. Frequently, the ER system is made of flat bladders that are openly connected with one another. Different forms of ER are shown in Figs. 6.1, 6.2 and 6.3.

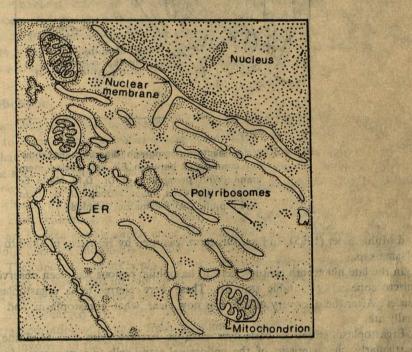
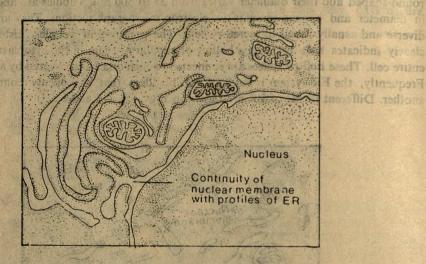


Fig. 6.3 Part of electron micrographs (diagrammatic) of neuroblast of cerebral cortex of the foetus of a mouse. Outer surface of ER is rough due to RNP granules. Cytoplasmic matrix is rich with polyribosomes. (Redrawn from De Robertis et al., Cell Biology, 1970.)

The number and position of strands of ER vary. For example, in meristematic cells, branches of the system pass right up to the plasmalemma and are parallel to the cell surface. The ER is intimately associated with the nucleus. There is an open connection between the hollow ER system and the lumen of the nuclear double membrane (Fig. 6.4).

Under an electron microscope, hollow spaces in the ER system seem to be mass-deficient. It is likely that they are filled with a kind of serum referred to as enchylema by Weyssling



are frequently against in parasi-

Fig. 6.4 Part of electron-micrograph (diagrammatic) showing connection between ER and nuclear membrane. (With permission of Dr. D.M. Mann, Ed. Scope Publications, Scope Monograph on Cytology, Upjohn Co., Kalamazoo, Michigan.)

and Muhlethaler (1965). In fact, this term was used hy Hanstein in 1880 to denote a kind of plasma sap.

In the late nineteenth century, certain basophilic regions had been observed under a light miscroscope, after suitable staining. These were referred to as 'ergastoplasm' or 'accessory nuclei'. After the discovery of ER, it is now clear what ergastoplasm and accessory nuclei really are.

Ergastoplasm stores the basic dyes such as haematoxylin and toluidine blue. They are particularly characteristic of the endocrinal gland cells (e.g. pancreas). In plant cells, however, they are very rarely observed in this characteristic manner. Under the electron microscope, ergastoplasm is seen as an aggregation of ribosomes which are situated on the parallel lamellae of the stacks of ER. Ribosomes may also show themselves as accumulating freely in the ground-plasm. Its basophilic property is due to the presence of RNA in ribosomes.

Accessory nuclei are actually diffused zones of cytoplasm that react with nuclear stains. Wrischer (1960) reported accessory nuclei in the short apex of Elodea after anoxia. They were also observed by Gavaudan et al. (1960) in the root tips of hyacinthus and by Falk (1962) in young sieve tubes. They consist of concentric rings of lamellae of ER. Formation of these rings is owing to insufficient supply of oxygen during the process of anaerobiosis.

Myccid bodies are the specialisation of ER. They are found in pigmented epithelial cells of the retina and are constituted of compactly arranged vesicles and tubules. They do not contain ribosomes, i.e. they are a part of smooth ER. They are sensitive to light and probably play some role in photoreception.

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## **Functions of ER**

### Intercellular Transport

tra margin of vesicles move come rections count the The form and distribution of the capillary system is indicative of the role of ER in migration and distribution of matter within the wall. Whenever the matter is consumed fast (e.g. in the synthesis of cell wall), capillaries of ER are seen running parallel to the sites of consumption. The cells of a tissue are in communication with each other through the plasmodesmata. Individual strands of ER pass through these plasmodesmata and whole bunches of strands through the pores of sieve plates. The cytoplasm of sieve tubes is full of canals of ER. These canals run parallel to the direction of the movement of the stream-assimilation products. All these observations lead to the conclusion of the role of ER in intercellular transports. It seems that chemical forces are involved in the transport and molecules glide along the surface of the membrane of the ER. This movement is probably like that in the ion bladder where attractive forces are involved and which move quickly along the wall. coryme and mergodites may be tran acted by the En or the region of wall-youthesis.

# Cellular Metabolism

The ER has an important role in the synthesis of assimilates. Its membranes offer an increased surface to the metabolic activities taking place within the cytoplasm. It has been demonstrated that isolated ribosomes can synthesise proteins from amino acids in vitro. This means that the seat of protein synthesis must be the ER where the ribosomes are situated. It is, however, not yet known for certain whether the hollow ER system provides the necessary amino acids or whether it translocates protein molecules that are synthesised. But it is obvious that in both the cases molecules would have to pass through the membrane of the reticulum.

Proteins that are formed in polysomes enter the cisternae of the ER and then move towards the Golgi complex and finally pass on to the condensed vacuoles situated near the Golgi complex. The condensation takes place in these vacuoles, whose contents then become concentrated by loss of water and interaction of proteins with other compounds. Consequently, there is formation of secretory granules. The vacuoles then move to the cell surface and granules are discharged by reverse pinocytosis. Autoradiographic studies show that there is direct transportation of proteins from ribosomes to the ER.

The ER is also involved in the synthesis of carbohydrates and enzymes that are proteinaceous in nature. Glycogen is formed in its caverns in the liver. Zy.nogen granules are produced in the pancreas. Vogel (1960) observed plastid-like bodies coated with membranes of ER in the glandular hairs of leaves of the insectivorous plant Pinguicula.

# Intercellular Impulse Conduction

vericles or a clear eleananth med by waginstich According to Porter (1956), the sarcoplasmic reticulum in striated muscles is a specialised arrangement of ER and it perhaps permits the transmission of impulses or excitations intercellularly deep inside from the cell surface in the myofibrils.

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# Behaviour of ER During Cell Division

1. During cell division, the nuclear membrane breaks up into pieces before the onset of metaphase and finally disintegrates into small vesicles (Moss, 1958, 60). As the metaliver hour opened by missing of committages which when collect microses by it is now well

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phase starts, these vesicles move towards the spindle poles where they cannot be distinguished from the elements of the ER. Both the elements of the reticulum and fragments of vesicles move to the regions around the chromosomes which are now grouped at the poles. Most of the elements of the reticulum either join or fuse around each group of daughter chromosomes and thus forming a new nuclear membrane. These observations indicate that ER plays a very important role in the formation of a new nuclear membrane during cell division.

2. It is now well known that in plants, the Golgi complex plays a major role in the formation of cell plates in cytokinesis. Bits of ER are also involved to some extent, in the cell plate formation. It is assumed that those elements that are not concerned with the formation of the nuclear membrane, move down into the interzone and come to the equator of the spindle. At this region, the advancing margins reticulate and a lattice of microtubules is formed. The cell plate is the first to appear in this lattice.

It is likely that in the formation of the secondary cell walls in plants, the required enzymes and metabolites may be transported by the ER to the region of wall synthesis.

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- 3. Certain evidences have led to the suggestion that the ER has its contribution to the development of the amphibian embryo.
- 4. The ER gives additional mechanical support to the colloidal structure of cytoplasm.

# Origin

The ER is an organeile which is most variable in the cell. In very young meristematic cells, it is found moderately while in growing cells and mitosis, its development is strong. In growing cells, it is possible to study the growth of branches and ramification out of the extant reticulum and to observe the manner in which vesicles are separated off and how they grow into strands. It may be thought that the strands that were taken over from the mother cell at the time of division might have given rise to all parts of the system of the ER. According to Palade (1956), here the problem is to find out whether initials of the reticulum can originate from invagination of the plasmalemma. In this connection, Wyssling and Muhlethaler (1965) have concluded that the ER is a system independent of the plasmalemma.

We have already seen that the ER and the nuclear membrane are directly connected to each other. In fact, the latter is a part of the former. The present evidence suggests that it is more probable that the nuclear membrane is the ontogenic source of the ER rather than cytoplasm itself. Buitrose (1963) observed that the perinuclear spaces in the nuclei of cells of wheat endosperm were markedly large. These spaces were found to be equipped with vesicles of nuclear plasma formed by evaginations of the inner nuclear membrane. The contents of these vesicles thus function as the cytoplasmic groundplasm. When the vesicles grow, the perinuclear space breaks up into septae and caverns characteristic of ER.

# Microsemes

Claude (1941) was the first to isolate small particulate components of the cytoplasm from liver homogenates by means of centrifugation. These were called microsomes. It is now well

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known that these fractions consist of fragments of the membrane of ER to which the ribosomes (previously called smail granules) are attached. The microsomal fraction as well as ribosomes are rich in RNA.

After claude isolated microsomes, Brachet and his associates put forward the idea that these structures were probably involved in protein synthesis. Experimental evidence indicates that the initial site of incorporation of labelled amino acids into cytoplasmic protein is the ribonucleoprotein granule component of the microsomal fraction (Fig. 6.5).

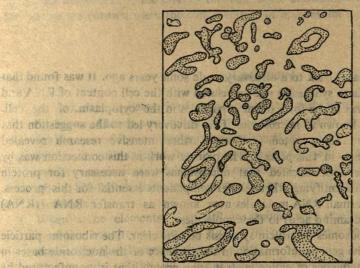


Fig. 6.5 Diagram of the electron-micrograph showing microsomes. Dots on the amplication to belowing linewes surface denote ribosomes. (Redrawn these and the value value of the Wilson and Morrison; Cytology, comme a second Thousand and the 1966.)

and 303 1 30.16). The decimined of the rain The term microsomes is frequently used when cytoplasmic structures are described since it is found to be convenient to apply it to the type of material obtained in the highest fraction when cells are disrupted and separation of their contents is effected through ultracentrifugation. It should be remembered that microsomes have no existence as separate structures or cell organelles. They are, in fact, small spherical vesicles formed as a result of disruption of ER. The microsomal fraction of homogenized cells may have ribosomes with it.

Of the total mass of the cell, 15 to 20% is constituted by microsomes. This part contains 50 to 60% of the RNA of the cell. This RNA actually corresponds to the ribosomes. Microsomes have a high percentages of lipids which include phospholipids, inosital, acetyl phosphotides (plasmalogens) and gangliosides. The phosphotides and the state of the

Of the microsomal enzymes, there are a group of stearases and NADH-cytochrome c reductase. The latter is often employed as a marker of the microsomal membrane. Its exact function is, however, not known. NADH-diaphosphorylase, glucose-6-phosphotase and Mg++ activated ATPase enzymes are also present in the microsomal membrane. It has been observed that microsomes exhibit a small amount of respiratory activity in which DPN- and TPN-linked cytochrome reductases and cytochrome b (extra-mitochondrial cytochrome) are involved. These can transfer electrons to molecular oxygen. However, in most cases, there is no proof that microsomal respiration or electron transport is coupled with phosphorylation of ADP to yield ATP.

Microsomes perform different biochemical functions. They are involved in the biosynthesis of phospholipids, ascorbic acid and steroids and take part in a series of reactions that require NADPH<sub>2</sub> and O<sub>2</sub>. They have also a role to play at certain stages in the synthesis of glyceride, phospholipid, glycolipid and plasmalogen.

### Ribosomes

The history of ribosomes traces back to a discovery made some years ago. It was found that the capacity of different cells to synthesize was correlated with the cell content of RNA and most of the cellular RNA was in the form of small particles in the cytoplasm of the cell. These particles were then known as microsomes. This discovery led to the suggestion that the particles must have some role in protein synthesis. Further intensive research revealed the importance of ribosomes in this process. The pioneer work in this connection was by Zamecnik et al. (1956). This work indicated that microsomes were necessary for protein synthesis. They succeeded in identifying other cellular components essential for this process. These components included small RNA molecules now known as transfer RNA (tRNA) and enzymes which attached amino acids to these molecules.

Later on, the role of ribosomes in protein synthesis became clear. The ribosome particle coordinates translation of the genetic information in the sequence of the nucleotide bases in the messenger RNA to the sequence of amino acids in each protein that is manufactured by the cell. The messenger RNA (mRNA) is transcribed from the DNA molecule.

The first systematic work on ribosomes began in 1957 by several groups of researchers. The one led by Alfred Tissieres and James D. Roberts was a noteworthy one. In the beginning, the work was concentrated mostly on ribosomes of Escherichia coli. These are composed of two unequal subunits 30S and 50S (Fig. 30.16). The size is determined by the rate in terms of Svedberg units (S) at which a particle sediments when it is spun at a high speed in an ultracentrifuge. These subunits together constitute the functional units of 70S ribosomes in protein synthesis in the case of bacteria. It may be asked why this unit is not 80S (30S +50S). The reason is that the two S values are not additive since the shape of a particle influences its rate of sedimentation. In each of these subunits, protein constitutes about onethird of the total mass, the rest being RNA. The subunit 30S incorporates one 16S RNA molecule containing about 1500 nucleotides while 50S contains 235 molecules of about 3000 nucleotides and 5S-7S RNA molecules of 120 nucleotides. The subunit 30S includes either 19 or 20 different protein molecules. The other subunit 50S has apparently more than 30 protein molecules. Metal ions, principally magnesium ions, are responsible in keeping the two subunits together and also in maintaining their structure. Calcium, manganese and cobalt ions are also said to have a stabilising role. Ribosomes contain 40-60% RNA (rRNA) and the rest is protein. RNA makes the core while the protein forms the covering delivated ATP-accentayings are also proved in the interestant manufacture branch of the coil.

The bacterial ribosome is either of the prokaryote or eukaryote type. In fact, one of the distinguishing features of prokaryote and eukaryote cells lies in their ribosomes. The

complete ribosome in the former is 70S while in the latter it is 80S, the subunits being 40S American 221: 18and 60S respectively.

Ribosomes contain 40-60% RNA (rRNA) and the rest is protein. RNA makes the core

while protein forms its covering.

Views Nath (1970), Cell Statesty C. Cound and Co. New State. It is now known that most mRNA molecules are attached to many ribosomes during protein synthesis. A group of ribosomes to which a molecule is attached is called a polysome. The number of ribosomes in a polysome depends upon the length of the mRNA. These ribosomes are bound to the mRNA through some attachment sites in the smaller subunits. Polysomes are formed when the concentration of magnesium ions increases

#### SUMMARY

1. A reticulum or network of strands associated with vesicle-like bodies was observed by Porter, Claude and Fullam (1945) in the cytoplasm of cells. Porter and Kallman (1953) termed it as endoplasmic reticulum (ER).

2. It is made of elongated strands or trabeculae. At some places the strands form large vesicles or cisternae while parallel membranes are found at others. Small granules of about 100-150 Å diameter have been noticed in the narrow space between these two elements.

3. When some elements of the ER are studded with small granules called ribosomes, it is of the rough or granular type. The other type is the smooth or agranular type, marked by the absence of these granules.

4. The strands of the ER consist of double membranes and the space between them varies. The number and position of the strands also vary.

5. The ER and the nuclear membrane are directly connected to each other.

6. The ER is concerned with intercellular transport, cellular metabolism and intercellular impulse condition. It plays a very important role in the formation of a new nuclear membrane during cell division. Bits of the ER are also involved to some extent in cell plate formation.

7. Microsomes are fragments of the membrane of the ER to which ribosomes are attached. Both are microsomal fraction and ribosomes are rich in RNA. Microsomes have a high percentage of lipids. They are involved in the biosynthesis of phospholipids, ascorbic acid and are active in steroid biosynthesis.

8. Ribosomes contain 40-60% RNA and the rest is protein. RNA makes the core and protein forms its covering. They are the seat of protein synthesis.

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# 7. Mitochondria

# Historical Background

Mitochondria were first discovered by Kollikar in 1850. He observed them as granular bodies in a striated muscle cell. Thirty-two years later, Flemming showed that they were threadlike structures in many cells. He called them filia. In 1892, Altmann coined the term 'bioplasts' to denote mitochondria. He considered them as symbionts comparable to bacteria. In 1898, Benda referred to them as mitochondria. Michaelis (1900) was the first to stain them with the vital stain, Janus Green B. The fine structure of mitochondria was first described by Palade and Sjostrand using electron microscopy.

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As regards the function of mitochondria, Kingsbury (1912), regarded them as sites of cellular respiration. Hogeboom et al. (1948) confirmed this assumption by giving a convinc-

ing experimental proof.

In recent years, significant progress has been made in the study of the specific ultrastructural organisation of mitochondria with the aid of electron microscopy. It has been demonstrated that mitochondria contain a specific type of DNA that is different from that of the nucleus and that they possess their own machinery for protein synthesis. It has even been indicated that they perhaps participate in interference and differentiation.

# Morphology

Both plant and animal cells contain mitochondria that are barely visible under a highpowered microscope. They, however, reveal a highly complex structure under an electron microscope. The common form is oval with a 0.5-1.0  $\mu$  diameter. The length is usually twice the diameter but in some cases it may be 7.0  $\mu$ . The filamentous form is very thin with a  $0.2 \mu$  diameter, while the rod- and sphere-shaped ones are extremely thick, with a diameter of 2.0  $\mu$ . Filamentous mitochondria may break into granules which can reunite.

Since mitochondria are labile organelles, they are readily disintegrated by the action of fixatives. For their fixation, those methods are employed that stabilise the lipoprotein structure by prolonged action of oxidising agents such as osmium tetraoxide, chromic acid and potassium dichromate. For staining, iron haematoxylin and acid fuchsin are generally used.

### Volume-Shape Changes

It has been observed that mitochondrial movements are more pronounced during interphase than during mitosis. In cultured fibroblasts, continuous and sometimes rhythmic changes in volume, shape and distribution of mitochondria have been observed. The principal types of motion are agitation and displacement of mitochondria from one part of the cell to another. Sometimes, mitochondria are attached to the nuclear membrane at a place near the nucleolus.

### Localisation

Mitochondria are usually distributed uniformly throughout the cytoplasm. Sometimes, they gather around the nucleus or in the peripheral cytoplasm. They accumulate near the spindle during mitosis, and when the cell division is complete, they are distributed in almost equal quantities between the daughter cells.

In some cells, mitochondria move freely, carrying ATP where it is required, whereas in others they are located permanently near the region of the cell where more energy is in demand. They may orient themselves in a definite basalapical direction parallel to the main axis. These orientations are related to the submicroscopic organisation of the cytoplasmic matrix and vacuoiar system. The direction of the diffusion contents within cells may determine their orientation.

# Number In reconstructed proof, and the state of the stat

The number of mitochondria per cell depends upon the intensity of metabolism. Glandular cells usually contain more number of mitochondria than cells with a passive function. For example, the glandular cells of plant nectaries are very rich in mitochondria as compared to the cells of surrounding ground tissue (Eggmann, 1962). The number of mitochondria also depends upon their size. Thus, if they are larger in size, their number is fewer but equally effective as small ones with a large number.

# Structure of the stand and puts since adjusted mistron also familia biss tools also

Two membranes of about 60 Å thickness constitute a mitochondrion (Figs. 7.1 to 7.4). It contains two compartments or chambers. The outer chamber comprises the space between the two membranes. It is 40-70 Å in breadth and contains a watery fluid. The inner compartment is called the inner chamber or inner membrane space. It is bound by the inner membrane and filled with a relatively dense material called mitochondrial matrix. Both the membranes are about 60-70 Å thick and separated by a space (outer chamber) of about 40-80 Å. The inner membrane projects into the mitochondrial cavity forming a series of folds called mitochondrial cristae (Figs. 7.1 and 7.2). The mitochondrial matrix is usually homogeneous but in some cases, it may contain a fine filamentous material or dense small

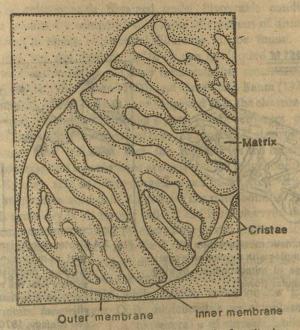


Fig. 7.1 Diagram of the electronmicrograph of a mitochondrion from the pancreatic cell (male) of Centroace. (Redrawn from de Robertis et al., Cell Biology).

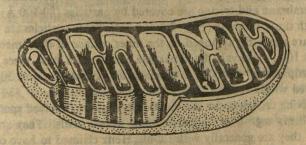


Fig. 7.2 Diagram showing the three-dimensional structure of a mitochondrion.

granules. These granules are regarded as sites for binding cations, especially Mg++ and Ca++. Water and small granules such as glycol and glycerol pass easily through all spaces of the mitochondrion. Both the membranes are permeable to paired ions such as Na+ and Clor NH4+ and C1-.

The mitochondrial cristae are generally incomplete septae or ridges so that the matrix remains continuous within the mitochondrion (Fig. 7.2). Recent researches indicate that the mitochondrial membranes may be more complex. The two outer layers may be of high electron opacity and the middle layer less opaque. The middle layer corresponds to the unit membrane structure. The outer and inner membranes and the cristae can be regarded as solid



Fig. 7.3 Diagrammatic representation of: Microtubules with oxysomes (upper). Structure of mitochondrion bearing microtubules (lower).

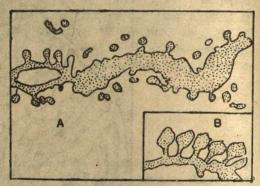


Fig. 7.4 Electronmicrograph (diagrammatic) of part of mitochondrion, A-Crista; elementary particles are seen on the surface near the matrix. B-Elementary particles attached to crista with stalks. (Redrawn from De Robertis et al., Cell Biology, 1970.)

molecular films having a compact molecular structure. The matrix is jel-like and contains highly-concentrated soluble proteins and smaller molecules. This double structure, i.e. solidliquid, is important when explaining some of the mechanical characteristics of a mitochondrion. The inner membrane and cristae are covered by particles (80-100 Å) that have a stalk connecting them with the membrane (Figs. 7.3 and 7.4). These particles are called elementary F<sub>1</sub> particles. They are regularly arranged on the inner surface of these membranes (Fig. 7.4) and spaced at about 100 Å intervals. Each particle consists of a base piece, a stalk and a headpiece. The headpiece is 75-100 Å in diameter and the stalk about 50 Å in length. The particles are contained within the thickness of the inner membrane.

Mitochondria having cristae occur in animal cells that are involved specially in intensive respiratory metabolism, e.g. liver, pancreas and kidney cells. They are not common in plants. In the latter, they are generally with tubuli. Heitz claimed to have observed a special type of mitochondrial organisation in fungi where there was aggregation of tubuli at both the poles of the elliptical organelle. Moore and McAlear (1963) studied 50 different species of fungi. They noticed a large variety of forms with tubuli, sacculi and cristae. It seems that the degree of intensity of function determines the organisation of the mitochondrion. Hence, during the development of the cell, its external form as well as internal structures may assume different and changing forms.

### **Functions**

The outer membrane of the mitochondrion may be regarded as the boundary of the cytoplasm and the inner membrane as the boundary of the chondrioplasm. If an invitro study is made of reactions of mitochondria obtained under favourable conditions with intact membranes and the results are compared with those of reactions of disintegrated fractions consisting of membranes in pieces, no essential difference will be found. This means that mitochondrial enzymes are not present in the stroma but located in the inner membrane. The reason for this localisation is that the inner membrane has remarkable increase of its area in the form of cristae. Maclennan (1970) and Green and Baum (1970) have described the mitochondrial function in detail. They have suggested that the electron-transfer chain or respiratory chain piece is a complex containing a part of the enzymes of the respiratory chain. The headpiece and stalk do not take part in the process of electron transfer.

In vitro experiments have indicated that the enzymes of the tricarboxylic acid cycle (Kreb's cycle), flavoproteins and cytochromes are present in mitochondria (Estabrook and Holowinsky, 1961). Succinic acid and dehydrogenase can be detected very easily. These facts indicate clearly that respiration centres in the cell are the mitochondria. It is for this reason that their presence is necessary in all aerobic cells. It has been observed that enucleated cells

or nucleus-free cell components obtained by microsurgery still continue to respire.

Although the sequence of reactions in the Krebs cycle was known by 1930, its cellular location was still unknown. The first report of particulate succinoxidase in higher plants was published in 1939 and 1940 but the significance of its particular nature was not still recognised. Goddard and Stafford (1954) while defining mitochondria gave precedence to the functional characters. According to Opik, mitochondria are cytoplasmic organelles with dimensions ranging from less than 1  $\mu$  to several  $\mu$  of variable labile shape. They have a smooth outer membrane and a highly infolded inner one. They are composed predominantly of lipoproteins, with small amounts of specific RNA and DNA. They are capable of carrying out certain biochemical reactions, notably the Krebs cycle, electron transport via the cytochromes and oxydative phosphorylation. The enzymes necessary for Krebs cycle are present in the matrix of mitochondria.

### Krebs Cycle

During the process of respiration, glycolysis occurs and leads to the formation of pyruvate (Fig. 7.5). By 1948, it was discovered that mitochondria in animals are the carriers of the Krebs cycle for complete oxidation of pyruvate (Figs. 7.6 and 7.7). At that time, the mitochondrial role in plant cells was doubtful. Subsequent researches showed convincingly their

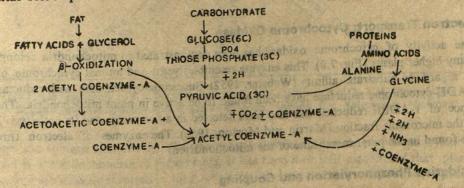


Fig. 7.5 Glycolysis.

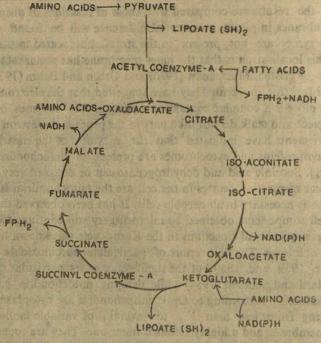


Fig. 7.6 Krebs cycle and related reactions.

participation in the Krebs cycle in plant cells also. Claude rightly spoke of mitochondria as the "power plants of the cell" since in the Krebs cycle, which forms an important phase of cell respiration, most of the energy is actually released. The removal of a hydrogen atom from a metabolite and its ultimate union with oxygen nets three molecules of ATP, which is the immediate source of energy for most vital processes.

Mitochondria of higher plant cells readily oxidise NADH and NADPH at a lower rate. This is also true in the case of intact, tightly-coupled preparations (Ikuma and Bonner, 1967). It is in contrast to mitochondria of animal cells where oxidation of only reduced coenzymes takes place when damaged.

# **Electron Transport: Cytochrome Oxidase**

The activity of cytochrome oxidase has been demonstrated in mitochondria belonging to many higher plants (Fig. 7.8). This enzyme resembles yeast and animal cytochrome oxidase in regard to substrate affinity (Webster, 1952). Succicinic-cytochrome with reductase and NADH-cytochrome reductase have been found to be active in plant mitochondria. The presence of NADPH reductase has also been detected in a few cases, and its activity is higher in the microsome fraction (Martin and Morton, 1956). The enzymes of electron transport are found in the inner membrane of the mitochondrion.

# **Oxidative Phosphorylation and Coupling**

Freebairn and Remnart (1957) and Zelitch and Barber (1960) have worked on mitochondria

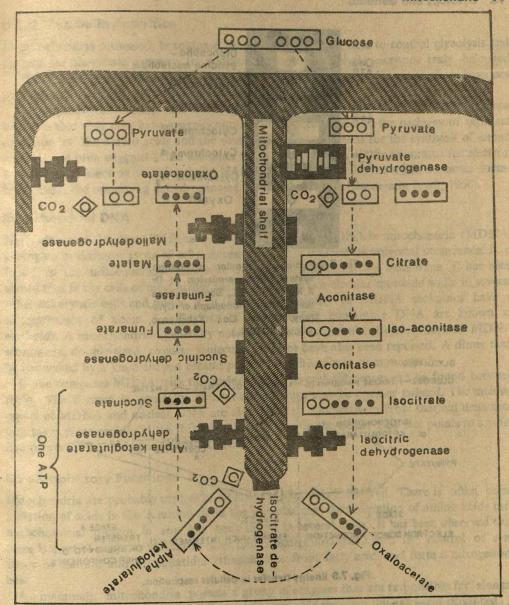


Fig. 7.7 Aerobic respiratory path in the mitochondrion. (With permission of Dr. K.M. Mann, Editor, Scope Publications, Scope Monograph on Cytology, Upjohn Co., Kalamazoo, Michigan, USA.)

in cabbage and spinach respectively. Their work indicates that the oxidative phosphorylation takes place in the mitochondria of these plants. It has been now well established that plant mitochondria are actively involved in the Krebs cycle, electron transport and oxidative phosphorylation (Fig. 7.9) at higher rates. There is accumulation of ATP molecules during cellular respiration. The mitochondria collect at places where the requirement of energy is

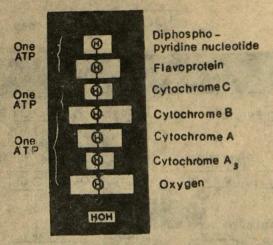


Fig. 7.8 Hydrogen transfer (oxidation) system. (With permission of Dr. K.M. Mann, Ed., Scope Publications, Scope Monograph of Cytology, Upjohn Co., Kalamazoo, Michigan, USA.)

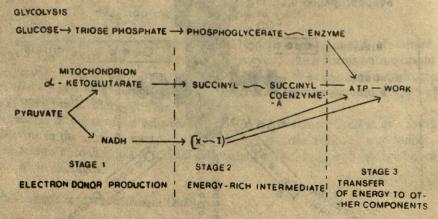


Fig. 7.9 Energy transfer in cellular respiration.

high. Water and ATP are sequezed out due to the membrane contraction and increase in the internal hydrostatic pressure of the mitochondrion. Hence, the ATP concentration decreases and there is relaxation of the mitochondrial membrane. The swelling of mitochondria is caused by the thyroid hormone thyroxin while ATP is responsible for contraction.

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# β-Oxidation of Fatty Acids

It is not yet certain whether the  $\beta$ -oxidative enzymes are present in the mitochondria of higher plant cells.

### Other Metabolic Activities

If mitochondria possessed, hexokinase they would have been able to control glycolysis and the pentose phosphate pathway. In addition, if other glycolytic enzymes truly belonged to the mitochondria, they would have also assisted in glycolysis. However, there have not vet been any intensive and critical researches in this connection.

The enzymes transminases and dehydrogenases are present in mitochondria. Their presence indicates that they may have a significant role in the Krebs cycle in the pathway of ultimate oxidation of the amino acids and the source of carbon skeletons for the synthesis of amino acids. It has been suggested that the inner membrane possesses all the apparatus for electron transfer from (electron transfer chain) NADH or succinate to oxygen while the outer membrane bears the remaining dehydrogenases of the Krebs cycle (Bachmann et al., 1966).

#### Mitochondrial DNA

Nass, Nass and Afzelius (1965) have shown the presence of DNA in mitochondria (MDNA) belonging to different types of cells. Recent researches indicate its universal occurrence. It is found in the mitochondrial matrix or it may be attached to the membrane. It has been shown that in the cells of most higher animals, MDNA is a circular molecule while in several other eukaryotic cells and plants, it is linear. A majority of MDNA molecules have a circumference of about 5 \u03c4. Regions of mitochondria containing DNA are known as nucleoids. A mitochondrion generally possesses 2 to 3 nucleoids. Besides normal MDNA monomers, double-sized circular molecules (dimers) have also been reported. A dimer may be connected by a knob-shaped central point to another dimer or monomer.

If one compares MDNA with the nuclear DNA, several differences can be found between them. The former is generally circular and its rings may be open or twisted. The melting points of MDNA and nuclear DNA are also different. The recovery from thermal denaturation takes place rapidly in the former while it is slow in the latter. This fact points to a more homogeneous base composition in MDNA.

# **Non-Respiratory Functions**

Mitochondria are probably centres of amino acid synthesis in the cell. There is often accumulation of acids in the Krebs cycle in the vacuolar sap. Incorporation of amino acids into mitochondrial protein in situ has been shown in several cases. It has been observed that there is a set of enzymes present in most mitochondria concerned with the control of synthesis of lecithin and phosphatidyl ethanolamine from fatty acids and form a nitrogenous base.

In mammals, mitochondria possess a group of enzymes that are responsible for elongation of fatty acids by adding acetyl CoA and afterwards reducing the keto-acid produced.

### Structural Changes

Mitochondria undergo structural changes in various tissues that are ageing. The maximum development of mitochondrial cristae in the leaves of Elodea and Chrysanthemum coincides with the maximum photosynthetic activity. The activity of cristae diminishes in senescence (Burat and Lance, 1958). Cells involved in active transportation acquire highly cristate mitochondria. This is also true in the case of various types of secretory cells (Luttage, 1966). In nectaries, the density of cristae becomes increased during the period of secretion but again decreases (Schnepf, 1964). Tapetal cells (Heslop-Harrison, 1963), phloem companion cells and filiform apparatus of synergids (Jensen, 1965) are all characterised by a large number of highly-cristate mitochondria. Following are a few examples illustrating changes in the mitochondrial structure in relation to the age of the tissue. They also indicate the degree of activity of mitochondria as a result of such changes.

- 1. In meristematic cells, the mitochondria are immature and therefore possess a few cristae. The respiration is partly fermentative.
- 2. Growth and differentiation are accompanied by an increase in the mitochondrial oxidative activity resulting from the synthesis of mitochondrial enzymes as is seen from an increase in the number of cristae. The Krebs cycle and cytochrome oxidase pathways are dominant in the respiration of actively growing tissues.
- 3. In mature tissues, a considerable proportion of respiration may proceed via PPP and perhaps also through soluble oxidases. This means that there is some decrease in the mitochondrial activity. In mature tissues, there is a correlation between high metabolic activity and highly-cristae mitochondria.
- 4. In senescence, there is decrease in mitochondrial activity, which appears to be controlled by chemicals in the ground cytoplasm. During the process of ageing, there is often dilation of cristae. In the extreme stages of senescence, there is disorganisation of the mitochondrial structure.

### **Biogenesis**

There are three main theories regarding the origin of mitochondria: (i) origin from various cell membranes, (ii) formation by division of parent mitochondria, and (iii) a de novo origin.

### From Various Cell Membranes

There is a great similarity between the outer membranes of the mitochondrion and the endoplasmic reticulum. Experiments using radioactive choline and leucene indicates that the outer membrane is more active in phospholipid synthesis while the inner membrane is predominantly concerned with protein synthesis (Bucher, 1968). These facts suggest that the outer membrane of the mitochondria may have some relationship with the other intercellular membranes. Several authors have observed continuities of mitochondria with the nuclear envelope, endoplasmic reticulum and even with the plasma membrane (Robertson, 1961; de Robertis and Bleichmar, 1962).

Robertson (1961) has demonstrated that the mitochondria in muscle cells originate from an infolding of the plasma membrane into which a projection passes from an adjacent part of the membrane. The result is a double-membraned vesicle. It was thought that the mitochondria are formed when the ingrowth of cristae from the inner membrane takes place. However, the fact that the outer and inner membranes of the mitochondrion differ in their physical and chemical properties, suggests that the origin from a common membrane is not probably possible. There has been also a suggestion that mitochondria perhaps arise from the Golgi complex but there is no convincing proof of this.

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# By Division of Parent Mitochondria

It has been demonstrated, using time-lapse cinematography, that mitochondria gradually elongate and then fragment into smaller mitochondria. This was verified by Luck (1963, 65) in Neurospora. He inferred from his observations that the mitochondria had probably divided and grown by the addition of new lecithin molecules to the existing framework of the mitochondrion.

# De Novo Origin

Several authors support the de novo origin of mitochondria. Harvey (1953) was the first to suggest it. He centrifuged eggs of sea-urchin into two halves. The halves containing the nuclei gave rise to mitochondria although they were devoid of them (mitochondria). De novo origin of mitochondria has also been reported in chick embryos, Neurospora and guppy fish. It seems that there may exist a relationship between growth and division of existing mitochondria and de novo synthesis. According to Atardi and Atardi (1967), mitochondria perhaps release a messenger RNA which then enters the endoplasmic reticulum where it forms the membrane protein with the help of ribosomes. This would lead to the de novo synthesis of mitochondria.

It has been suggested that mitochondria may arise from chloroplasts. Of course, this cannot be applied to animal cells. to the standard between the case of the standard the stan

# Evolutionary Origin of Mitochondria Study The super greather is allost, with cutter security words which is a relative

# Prokaryotic Origin

Recently, it has been established that mitochondria contain all the necessary machinery for synthesising proteins from amino acids. Ribosomes have already been reported in them. These are, however, smaller than cytoplasmic ribosomes and more akin to bacterial ribosomes. In mitochondria chloramphenicol inhibits protein synthesis. Such an inhibition also occurs in bacteria while cytoplasmic protein synthesis is not affected.

The old view that mitochondria represent the symbiotic organisms living in association with higher cells has been reviewed. It is now stated in terms of modern cell and molecular biology. There are many homologies between bacteria and mitochondria. For example, the general dimensions are nearly the same. Rod-shaped bacteria are similar in shape to several types of mitochondria. There are also similarities in the localisation of the respiratory chain. In bacteria, there is localisation of the electron transport system in the plasma membrane, very similar to the inner membrane of mitochondria. In certain bacteria, there are membranous projections that extend from the plasma membrane forming what are known as mesosomes. Such membranes contain the respiratory chain. Since the outer membrane of the mitochondria is similar to the endoplasmic reticulum, the inner membrane and matrix may represent the original symbiont and be enclosed within a membrane of cellular origin. As already mentioned, the mitochondrial DNA is generally circular and so also are the chromosomes of prokaryotes. It replicates and divides into several circles which may be seen in a single mitochondrion.

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In the symbiont hypothesis, the host cell is supposed to be an anaerobic organism deriving its energy from glycolysis, which takes place in the cytoplasmic matrix. The bacterialike parasite supposedly contains the respiratory chain and the Krebs cycle is considered to have occurred therein. This means that it was capable of carrying on respiration and oxidative phosphorylation. Moreover, the presence of DNA polymerase, RNA polymerase, tRNA activating enzymes, ribosomes and amino acid incorporating activity in mitochondria show that there exists an autonomous protein synthesis machinery in mitochondria similar to that of free-living organisms. In the case of plant cells, this hypothesis is more attractive because the parasite then can be considered as the chloroplast, that is, an autotrophic microorganism capable of transforming light energy. In connection with this hypothesis, it should be borne in mind that the amount of MDNA is not sufficient to code all the required proteins of the mitochondrion.

### SUMMARY

- 1. Mitochondria occur in the cytoplasm of both plant and animal cells. Palade and Sjostrand (1º40, 50) were the first to describe their fine structure using an electron-microscope. The common form is oval with a diameter of 0.5  $\mu$ . The filamentous form is very thin. There are usually distributed uniformly throughout the cytoplasm. They accumulate near the spindle during mitosis.
- 2. A mitochondrion is constituted by two membranes and contains two chambers. The outer chamber consists of the space between the two membranes and contains a watery fluid. The inner chamber is filled, with mitochondrial matrix which is a relatively dense material. Its inner membrane forms a series of folds projecting into the cavity. These are called mitochondrial cristae. The inner membrane and cristae are covered with particles consisting of a head and a stalk connecting them with the membrane. Mitochondria having cristae occur in animal cells which are involved especially in intensive respiratory metabolism.
- 3. The enzymes of the Krebs cycle, flavoproteins and cytochromes are present in mitochondria which are the centres of respiration in the cell. The enzymes necessary for the Krebs cycle are present in the mitochondrial matrix. The enzymes involved in electron transport are found in the inner membrane. Mitochondria are also involved in oxidative phosphorylation. They are probably centres of amino acid synthesis in the cell.
- 4. DNA is found in the mitochondrial matrix. It is a circular molecule in the cells of most higher anit als, and linear in several other eukaryotic cells and plants. There are several differences between mitochondrial DNA and nuclear DNA.
- 5. There are three principal views regarding mitochondrial origin. As regards the evolutionary origin of mitochondria, the prokaryotic origin and symbiont hypotheses have been put forward.

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# 8. The Golgi Complex, Spherosomes and Lysosomes

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# Golgi Complex

### Historical Background

In 1898, the Italian microscopist Golgi described certain previously unknown bodies in the cytoplasm of nerve cells of barn owls and cats using a special stain developed by him. He described these bodies as extremely small structures that appeared to be made of minute plates and threads. The structure was called the Golgi apparatus. In fact, the Spanish histologist Cajal had observed these bodies in silver-stained cells several years earlier but had not reported his observation because, as one of his later writings indicates, "the confounded reaction never appeared again".

In 1900, Holmgren described a system of clear canals which he termed trophospongium. Probably he was referring to the endoplasmic reticulum. In 1917, Gatenby pointed out some similarities between the Golgi apparatus of vertebrates and the dictyosomes of invertebrates. The vacuome theory in respect of the Golgi apparatus was propounded by Accoyer (1924) and Parat and Painleve (1924). According to this theory, the vacuome and chondriome are the two fundamental units of the cell. The former consists of vacuoles or a canalicular system. The Golgi apparatus and trophospongium were regarded as artifacts.

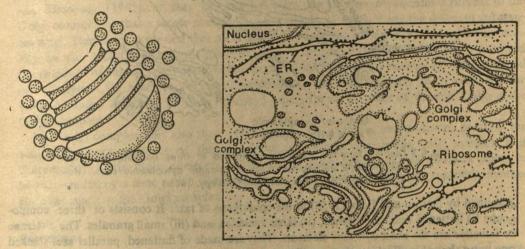
Baker (1951, 53) used the term lipochondrium to denote the Golgi apparatus as he assumed that it contained a lipid. Structures similar to the Golgi apparatus were also observed in plants (Perner 1958), and botanists termed them as dictyosomes. Fungi were, however, regarded to be devoid of the Golgi apparatus. Recently, they have been reported in Ascomycetes, Saccharomyces (Moor and Muhlethaler, 1963) and Neobulgaria (Moore and McAlear, 1963a). Nowadays, the term Golgi complex is preferred to the Golgi apparatus.

# **Light Microscope Structure**

The Golgi complex in animal cells as studied under a light microscope can be at least differentiated into an outer osmiophilic component and an inner osmiophilic component. In nerve cells, the Golgi complex consists of osmiophilic granules (Thomas, 1960) rather than lamellar stacks.

#### Ultrastructure

In recent years, studies with the aid of electron microscopy have shown that the Golgi complex possesses a generalised pattern consisting of a system of membrane-bound vesicles of various sizes accompanied by smooth membrane elements arranged in a more or less parallel manner (Figs. 8.1 to 8.3). The present evidence indicates that the Golgi complex is a separate organelle and not a part of the endoplasmic reticulum. The facts that support this view are: (i) the membrane system does not have associated granules; (ii) the general morphology of the Golgi complex is very similar in diverse types of cells; (iii) though the membranes contain elements of the endoplasmic reticulum, they are much thicker; and (iv) the Golgi complex seems to be specifically able to reduce osmium tetraoxide. There is some evidence to indicate continuity between the smooth membranes of the Golgi complex and certain elements of the endoplasmic reticulum.



showing its three-dimensional structure.

Fig. 8.1 Diagram of Golgi complex Fig. 8.2 Diagram of electronmicrograph of a plasma cell. Golgi complex is near the nucleus, It is formed by flat cisternae and large and small vesicles. It is surrounded by granular ER (Redrawn from de Robertis et al., Cell Biology, 1970.)

The Golgi complex is a series of double membranes concentrically bent. Just as the membranes of the endoplasmic reticulum enclose sublight-microscopic spaces, in the Golgi complex also there are minute spaces (vesicles) between the membranes. There are inflated extensions along the periphery of the vesicles. The small round vesicles may join together and form large vesicles. Even under an electron microscope, no network is visible anywhere and hence, it is not proper to use the term dicytosomes. The controversy in regard to whether the Golgi complex is a network of an accumulation of small vacuoles arose from the fact that there appeared under the light microscope either a coagulum of osmiophilic lamellae (network) or microvacuoles which could be resolved. Hibbard (1945) has stated that in a living cell the typical network of fixed micropreparations has never been observed.

A detailed description of the Golgi complex as studied under an electronmicroscope was sinteraldehyde, followed by negative meining

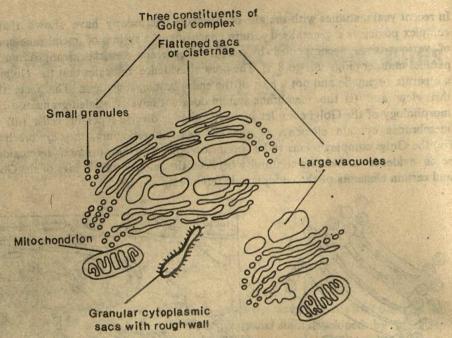


Fig. 8.3 Diagram from Golgi complex composed of three constituents. Two mitochondria are also seen besides granular cytoplasmic sacs. (Redrawn from Wilson and Morrison, Cytology, 1966.)

described by Dalton and Felix (1953) in the epididymis of rats. It consists of three components: (i) flattened sacs or cisternae, (ii) large vacuoles, and (iii) small granules. The cisternae or lamellae are the most constant elements. They are made of flattened, parallel sacs stacked one upon the other. Each stack generally contains 3 to 12 cisternae. The cisternal unit in a section is seen as a pair of parallel membranes that are continuous at the ends. A thin cavity about 150 Å wide is enclosed by these membranes. The placing of the cisternal units is about 200 to 300 Å apart. The cisternae are generally slightly curved but they may be flat in some cases.

According to Mollenhauher and Whaley (1962), the Golgi complex is polarised in certain plant cells and has a 'forming face' and a 'maturing face'. The smooth membranes of the endoplasmic reticulum bud off vesicles which subsequently get arranged on the forming face of the stack. On the other side is the maturing face where the lamellae bud off secretory vesicles.

The large vacuoles are clear. They are usually found at the edge of the Golgi complex. They are modified and expanded cisternae in which the two membranes were widely separated so that the vacuolar space became enlarged.

The diameter of small vesicles is about 400 to 800 Å. They form an intimate association with the cisternae and may be continuous with the latter. They originate from the cisternae by the process of budding or pinching off.

In case of plant cells, to study the Golgi complex (dictyosomes), fixation is done using gluteraldehyde, followed by negative staining with phosphotungstic acid. It consists of

stacks of flattened and fenestrated cisternae from where many anastomosing tubules are given off. Their diameter is between 300 and 500 Å. Two kinds of vesicles-rough- and smooth-surfaced—are associated with these tubules. The rough-surfaced vesicles are spherical and about 600 Å in diameter and while the smooth-surfaced ones vary in size. There are parallel rods or fibres of diameter 70 to 80 Å between the cisternae.

# **Chemical Nature**

The histochemical study of the Golgi complex indicates the presence of lipids, proteins and glycoproteins in it. In various kinds of epithelial cells, alkaline as well as acid phosphatases appear to be concentrated in the Golgi complex. Schneider and Kuff (1954) established the presence of RNA, phospholipid and phosphatase in the Golgi complex. According to Deane and Dempsey (1945), it may be that there are two separate phosphatases present in the Golgi complex or that a single phosphatase is capable of hydrolysing the different substances used. They have suggested that all cells may possess sites of significant phosphatase activity in the Golgi zone at some point for some substrate. Allen and Slater (1961) observed the presence of thiamin pyrophosphatase in the Golgi region of a number of cells. Several other enzymes, for example, ATPase, 5-nucleotidase, etc. are also seen to be located in the Golgi complex. Bourne (1960) has furnished proofs for the presence of vitamin C also in the Golgi complex.

The Golgi complex may also show variations in its chemical composition because the visible secretory products of the cell appear to be formed first in the Golgi complex. It is characteristic of the Golgi complex to be devoid of ribosomes. The Golgi complex seems to be surrounded by a zone where ribosomes are apparently absent. Hence, no protein synthesis takes place in the Golgi complex.

#### **Functions**

It has been shown that there exists some relationship between the Golgi complex and secretion. It is seen that those cells which exhibit high secretory activity, particularly those secreting either proteins or complex polysaccharides, contain a very well-developed Golgi complex.

The work based on autoradiographs indicates that although proteins are synthesised in the endoplasmic reticulum, their condensation often takes place in the Golgi complex, resulting in the formation of secretory glanules or other cell products, e.g. many exocrine and endocrine secretions. In plant cells, when complete polysaccharides are synthesised, they are passed on to the cell via vesicles. In the glandular hair of insectivorous plants, e.g. Drosera, and outer root cap cells (Mollenhauer et al., 1961; Mollenhauer and Whaley, 1962), there exists a quantitative relationship between the secretion of mucilage and the number of Golgi complexes. The chemistry of mucilage indicates that the synthesis of further polymeric carbohydrates is perhaps carried on in the Golgi complex.

Zymogen granules are first formed in the Golgi complex and then they separate from it. They are surrounded by a membrane and stored in the cytoplasm as the final zymogen granules. They are now ready for their discharge into the lumen under the influence of a secretion stimulant. The role of the Golgi complex in the secretion process has been demonstrated in many gland cells, e.g. in human sweat glands, frontal lobe of the pituitary gland of rats and thyroid gland of salmanders to the appropriate the district of the appropriate and the

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It has recently been found, employing labelled glucose with tritium, that in rats the Golgi complex is the actual site where the carbohydrate is added to the protein. However, it must be remembered that the Golgi complex is not the only place for the formation of the side chains in glycoproteins and mucopolysaccharides. As soon as the protein backbone is formed, the side chain starts to form. The bulk of it is formed in the Golgi complex.

Enzymes including acid phosphatase have been shown to be active in the Golgi complex (dictyosomes) indicating their part in the reaction taking place within the Golgi complex.

The Golgi complex or one of its homologues in certain protozoa is present in the vicinity of the contractile vacuole. This means that the Golgi complex takes part in the intercellular water-transfer system. It also participates actively in the incorporation of substances from the environment. It is seen in the epithelial cells of the intestine where it appears enlarged because of fat or protein resorption.

#### **Cell Plate Formation**

It has been recently shown that the Golgi complex is involved in cell plate formation also (Fig. 8.4). The cell plate formation takes place during cytokinesis when there is fusion of the

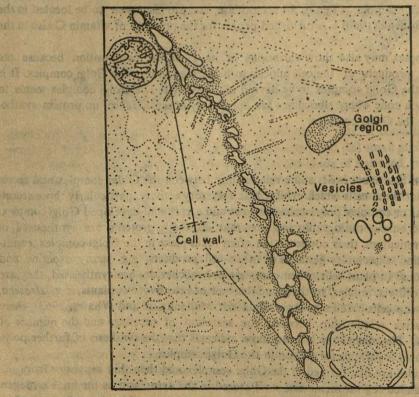


Fig. 8.4 Electronmicrograph (diagrammatic) of cell of spinach showing Golgi complex which is producing material of cell wall. Near its margin, vesicles are formed and some of them are transferred to the newly formed cell plate. They are united with the cell wall. (Redrawn from Ambrose and Easty, Cell Biology, 1971.)

material carried in the vesicles up to the site of its formation (Bajer, 1965). During cell plate construction, numerous dictyosomes cut off vesicles that serve to the cell plate region. These vesicles fuse and components of the new cell plate are released (Frey-Wyssling et al., 1964). Autoradiographic studies have revealed that during this time, pectic substances are probably produced by the Golgi complex. It is likely that the vesicles enter the region of the mitotic spindle at telephase. The small vesicles sometimes pass in rows between the radiating microtubules up to the area of the cell plate. The plate extends outward towards the mother cell. There is a concentration of vesicles and microtubules at its edge. The microtubules remain in this region and radiate backwards towards the disappeared poles of the spindle on each side of the plate.

# Cell-Wall Formation

It seems that the Golgi complex takes part in cell-wall formation also by contributing substances which are most likely hemicellulose.

### Origin

Examination of growing meristematic cells during the early stages shows increase in the number of Golgi complexes, while during differentiation, leading to mature cells, the number may decrease markedly. According to Moore and McAlear (1963), the Golgi complex is formed from the nuclear envelope in the exciple of the apothecium of the Discomycetes member Neobulgaria. Therefore, they think that the origin of the Golgi complex would be the same as that of the endoplasmic reticulum.

The Golgi complex does not appear as lamellar packets from the yery beginning but as a concentric lamellar system. These cap-shaped vesicles then open and become flat so as to form typical saucer-shaped lamellae characteristic of the Golgi complex. This means that the detached vesicles do not originate from the club-shaped strand ends but bud off from a circular saucer rim. Such a specific form of the Golgi complex is seen in the serial sections or in the surface view after freeze-etching.

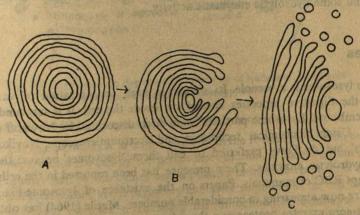


Fig. 8.5 Origin of Golgi complex. A-concentric double membrane. B-Opening of bilamellar circles. C-Formation of vesicles from the margin of saucer-shaped double membrane.

# Spherosomes made a making of the total the or que sale content of the total to the total of the

In 1880, Hanstein observed small, highly refractive bodies of a 'denser substance' in the cytoplasm of plant cells. He called them *microsomes*. These bodies can easily be observed under dark-field illumination as brightly shining objects and in phase contrast as black granules. Their diameter is 0.5 to  $1.0~\mu$ . Plant cytologists used them as convenient objects to study Brownian movement and plasma streaming in cells. These particles are about  $0.1~\mu$  in diameter. Today the term 'spherosomes' is preferred to 'microsomes'.

Fat dyes like Sudan III stain spherosomes like fat droplets. Since they show Nadi reaction (synthesis of indophenol blue from a naphthol and dimethyl-para-phenylene diamine through O<sub>2</sub> transfer), Perner (1958) concluded that spherosomes were not ergastic particles like fat drop lets but enzyme-active definite organelles containing cytochrome oxidase. It is, however, possible to show that the abovementioned reaction is catalysed by mitochondria and spherosomes act merely as selective storage sites for the stained reaction product indophenol blue.

A spherosome is surrounded by a unit membrane. It contains acid phosphatase. The presence of this enzyme suggests that the spherosome is probably active in lipid metabolism. However, spherosomes have not shown as wide a range of lytic action as lysosomes. They appear to act as storage bodies for similar types of enzymes.

There are two views about the development of spherosomes. According to one view, they develop first as precursors of reverse oil droplets and then bud off from the endoplasmic reticulum. They contain enzymatic proteins which can synthesise fats and oils. The buds, sometimes referred to as prospherosomes, develop further with an increase in the lipid content with a concomitant decrease in proteins. According to the other view, spherosomes are not precursors of fat or oil bodies since there is coalescence of spherosomes. Fat bodies have no membranes and they coalesce easily. Spherosomes do not contain any kind of natural fat. Whichever view may be correct, there is no doubt that spherosomes are the principal sites of lipid storage in plant cells. They are similar to lysosomes in that they possess a single membrane and some hydrolytic enzymatic activity.

# Lysosomes

Basically, the lysosome is a vacuole. It contains a high concentration of various enzymes required in the digestive processes carried on inside the cell. It is surrounded by a single outer unit membrane. Lysosome particles were first discovered by Novikoff (1961) and de Duve (1959). Using biochemical techniques and electronmicroscopy. Novikoff called them 'dense bodies' but de Duve preferred to call them 'lysosomes' because they were found to contain hydrolytic enzymes. Their presence has been reported in the cells of almost all animals, except RBC of mammals. Papers on the existence of lysosome-like structures in plant cells are now appearing in considerable numbers. Matile (1964) has observed that the fungus Neurospora excretes proteases in the culture medium for the extracellular digestion of proteins. These proteases are bound intracellularly to a particle fraction, which he thinks are probably lysosomes.

### Morphology

Generally, lysosomes appear as a dense body surrounded by a membrane. They usually vary in diameter from 0.4 to 0.8  $\mu$  but they may be up to 5  $\mu$  in mammalian kidney and extremely large in phagocytes. They are spherical, rod-like or irregular in shape and covered by a single-layered membrane made of lipoprotein. Their internal structure is variable because of their different functional activities. Some lysosomes are solid uniformly while others possess an extremely dense core. There may be still others having cavities or vacuoles with granular material. The lysosomal contents are denser as compared to those of mitochondria.

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### Types

There is considerable variation in regard to the mechanism by which the materials to be digested become enclosed inside a lysosome. In many cells, the Golgi complex produces primary lysosomes. These are small bodies. Their enzyme content is synthesised by ribosomes and it then gets accumulated in the endoplasmic reticulum. Subsequently, it penetrates into the Golgi complex wherein occurs the first acid-phosphatase reaction. The term secondary lysosome is applied when the enzymes and the material to be digested or already digested are present within a lysosome. There may be accumulation of large quantities of undigested or indigestable molecules in lysosomes. These are called residual bodies (Fig. 8.6). The autophagic vacuole cytolysosome or autophagosome is regarded as a special case in which lysosome contains a part of cell (e.g. mitochondria or portion of ER) in a process of digestion.

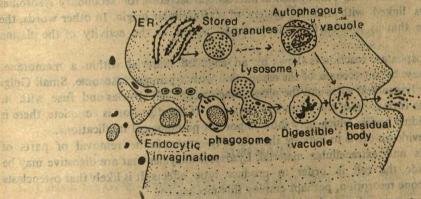


Fig. 8.6 Diagrammatic depiction of functions of lysosome. (Redrawn from Ambrose and Easty, Cell Biology, 1971.)

# Relationship of Lysosomes to Phagocytosis and Pinocytosis

Injection of the enzyme peroxidase in animals results in the formation of phagosomes, whose fate can be followed within the cell by the peroxidase reaction. If haemoglobin is injected, it is engulfed by tubular cells of the kidney and a positive phosphate reaction is shown by the phagosome. This reaction starts at the periphery and after sometime is carried to the interior. In addition, autophagic vacuoles surrounding the mitochondrial remnants and residual bodies with a layered structure may also be seen. This structure contains an undigested material which is probably lipid in nature. These different types of lysosomes indicate the variety of material digested and several ways of lysosome function.

#### Chemistry

Lysosomes possess a variety of enzymes (for example, proteases, ribonucleases, deoxiribonucleases, phosphatases, cathepsin, glucosidases and sulfatases) which break down all the principal constituents of living things, i.e. proteins, nucleic acids, organic-linked phosphates, polysaccharides and organic-linked sulphates. As most of these enzymes work more efficiently under a slightly acid constituent, they are together known as acid hydrolases. They do not contain oxidative enzyme, which is, however, present in mitochondria. This fact is suggestive of the idea that they represent a discrete group of cytoplasmic particles. When there is disruption of the membrane of lysosome, all the enzymes that are present are released and they become active simultaneously.

#### **Functions**

According to the present concept, primary lysosomes are regarded as a secretion product of the cell. The product, like other secretions, is synthesised by ribosomes. It first enters the endoplasmic reticulum and then reaches the Golgi complex for final packaging. A cell may produce different types of lysosomes, peroxisomes and several other secretion products by this mechanism. Hence, it is possible that there exists a kind of topological specificity in the endoplasmic Golgi system. Some suggest that glycoproteins are first produced in the Golgi complex and then migrate by way of small vesicles towards the lysosomes or the cell surface to form the extracellular coat. This system of intercellular secretion for secondary lysosomes is in some ways linked with another system of extracellular origin. In other words, the exoplasmic space that is formed by endocytosis is related to the activity of the plasma membrane.

A positive phosphatase reaction is shown by an engulfed material within a membrane. This reaction may be on account of the association with a primary lysosome. Small Golgi vesicles (primary lysosomes) surround the phagosome or engulfed vesicles and fuse with it. This results in the formation of the secondary lysosome. If the digestion is complete, there is formation of residual bodies which may show important pathological implications.

Considerable evidence exists to indicate the role of lysosomes in the removal of parts of cells, entire cells and extracellular material. Lysosome enzymes that are digestive may be discharged outside the cell in order to produce lytic effects. It is likely that osteoclasts associated with bone resorption, perhaps employ this mechanism.

The aforesaid account indicates that lysosomes perform very important functions. Although they are called 'suicide bags', they possess a built-in-mechanism that enables a cell to adapt metabolically to rapidly containing conditions in regard to oxygen concentration, food supply and other environmental factors.

# Origin of discharged in more arbitrary all yet the off many freehold in the

There are several views on the origin of lysosomes. Some think that they have a multiple origin depending either upon the tissue in which they are present or their function in a cell. Others suggest that a lysosome perhaps presents a pinocytic vacuole, meaning thereby an extracellular origin. It may carry on its enzymatic activities after becoming a part of the cytoplasmic machinery. Certain evidences are there to indicate the origin of lysosomes from the Golgi complex and that they represent zymogen granules. On the basis of present

data, it seems probable that the membrane might have originated from the Golgi complex and the enzymes from the activity of ribosomes.

Figure 8.7 depicts diagrammatically the functions of different organelles in an animal

cell.

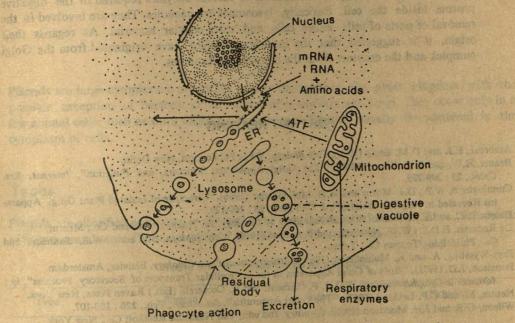


Fig. 8.7 Diagram showing functions of different organelles of an animal cell. (Redrawn from Ambrose and Easty, Cell Biology, 1971.)

### SUMMARY

- 1. Earlier, the Golgi complex or apparatus was thought to be present only in animal cells but recent research has indicated that it is present in plant cells also. It is a separate organelle occurring in the cytoplasm of cells and consists of a series of double membranes that are concentrically bent. There are minute spaces or vesicles between the membranes. The Golgi complex consists of flattened sacs or large internal vacuoles and small granules. It contains lipids, proteins and glycoproteins. Phosphatases, RNA, phospholipids, ATPase and 5-nucleotidase have also been
- 2. The condensation of protein granules, after their synthesis in the ER, takes place in the Golgi complex and results in the formation of secretory granules or cell products. Zymogen granules are first formed in the Golgi complex. Recent research indicates that the Golgi complex is involved in the intercellular water transfer system and cell plate formation. In rats, it is the actual site where carbohydrate is added to the protein.

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- 3. Spherosomes are small highly refractive bodies found in the cytoplasm. They are enzyme-active organelles containing cytochrome oxidase. They are surrounded by a unit membrane.
- 4. Lysosomes contain a high concentration of various enzymes required in the digestive process inside the cell. Basically, lysosomes are vacuoles. They are involved in the removal of parts of cells, entire cells and extra-cellular material. As regards their origin, it is suggested that the membrane might have originated from the Golgi complex and the enzymes from the activity of ribosomes.

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# 9. Plastids

Plastids are large cytoplasmic organelles present throughout the plant kingdom with the possible exception of bacteria, certain algae, myxomycetes and fungi. They occur only in a few animal cells, such as some flagellate protozoans. Plastids usually lie scattered in the cytoplasm of cells.

# Types

Plastids are classified according to the nature of the pigment contained in them. Sometimes pigments are absent. The main types of plastids are indicated in Table 9.1.

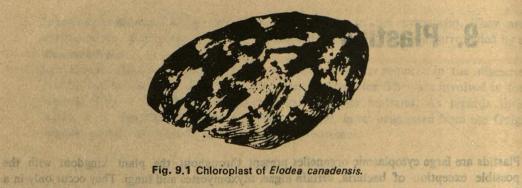
Table 9.1 Principal types of plastids

|    | Туре   | Pigment   | Source                                    | Function  |
|----|--|---|---|---|
| I. | Chromoplasts<br>(coloured)<br>Chloroplast<br>Phaeoplast<br>Rhodoplast    | Chlorophyll a and chlorophyll b Fucoxanthin Phycocrythrin | Higher green plants Brown algae Red algae | Photosynthesis  Absorbs light Absorbs light               |
| п. | Leucoplasts<br>(colourless)<br>Amyloplast<br>Elaioplast<br>Proteinoplast | None<br>None<br>None                                      | Some monocots -do- Seeds                  | Storage of starch<br>Storage of oil<br>Storage of protein |

In algae, pigmented plastids absorb light and transfer energy to chlorophyll a, which takes part in photosynthesis. Plastids present in blue-green algae are usually called *chromatophores*.

# Shape and Size

Plastids may be ovoid (Fig. 9.1), cylindrical or spherical in shape. They are the largest organelles present in the cytoplasm, their average diameter being 4 to 6  $\mu$  and thickness 1 to 3  $\mu$ .



few animal cells such as some lanellate pentagosas; a satida casally in sentimed in the

### Chloroplast

Chloroplasts vary in size, shape and distribution in different cells and species. However, they are relatively constant within the same tissue. The number of chloroplasts in higher plants is about 20 to 40 per cell. Their average diameter is about 4 to 6 \u03c4 and thickness 1 to 3 µ. They are either spherical, ovoidal, discoidal or club-shaped. Algal cells often have a single large chloroplast (Fig. 9.2) which may be in the form of a network, spiral,

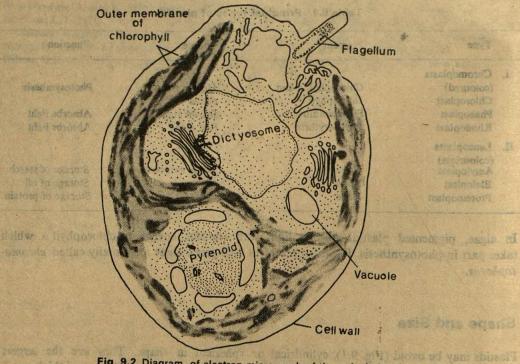


Fig. 9.2 Diagram of electron micrograph of the unicellular alga Chlamydomonos. Note the very large cup-shaped chloroplast.

band or star-shaped plate. When they are distributed homogeneously within the cytoplasm, they are rarely packed near the nucleus or close to the cell wall. Their distribution is largely governed by external factors such as light intensity. Their number is relatively constant in different plants. Haberlandt (1914) has estimated that there are about 4,000,000 chloroplasts per sq. mm in the leaf of Ricinus communis.

## Chemistry and the second of the following at

The chemistry of chloroplasts is far better known as compared to that of leucoplasts. Lichtenthaler and Park (1963) have given the chemical composition of spinach chloroplasts (Table 9.2).

Table 9.2 Chemical composition of spinach chloroplasts

| Moles |   | Molecular weigh |  |
|-------|---|-----------------|--|
| 115   | Chlorophylls (80 chlorophyll a+35 chlorophyll b)        | 103,200         |  |
| 24    | Carotenoids (7B-carotene, 11 lutein, etc.)              | 13,700          |  |
| 23    | Quinones (14 plastoquinone, 7 tochophenol, 2 vitamin K) | 15,900          |  |
| 58    | Phospholipids (26 phosphoglycerol, 21 lecithin, etc.)   | 45,400          |  |
| 72    | Digalactosyl diglycerides                               | 67,000          |  |
| 173   | Monogalactosyl diglycerides                             | 134,000         |  |
| 24    | Sulpholipids  | 20,500          |  |
| 7     | Sterols   | 7,500           |  |
| 100   | Unidentified lipids                                     | 87.600          |  |
|       | FOLHOLL Y LONG  | 494,800         |  |
|       | LIPIDS  | No.             |  |
| 1000  | N stome or protein                                      | 464,000         |  |
| 1690  | N atoms as protein                                      | ₩ 55            |  |
| 1     | Mn  | 336             |  |
| 6     | Fe C  | 159             |  |
| 3     | PROTEIN   | 46,500          |  |
|       | Minimum molecular weight per atom of Mn                 | 960,000         |  |

Table 9.3 gives the approximate chemical composition of isolated chloroplasts of higher plants.

The cell is first homogenised by a special procedure and then chloroplasts are isolated by differential centrifugation. About 80% protein is insoluble. It is intimately bound to lipids resulting in the formation of lipoproteins. A saturated protein has also been isolated and it has been noted that it may form 1:1 complexes with chlorophyll under certain conditions. Of the total proteins, 40% is accounted by this protein. Chloroplast enzymes constitute an important part of the remaining proteins. These enzymes may be soluble or go into the structure of the protein. Neutral fats, phospholipids, steroids and waxes belong to the lipid fraction.

Chlorophyll (Figs. 9.3A, B, and C) is one of the principal components of chloroplasts. It is an asymmetrical molecule, with a hydrophilic head comprising four hydraulic nuclei

Table 9.3 Approximate analysis of chromoplasts of higher plants (after Granick, 1961)

| Constituent   | % Dry wt   | Components Total Annua there  |
|---------------|--|---|
| Proteins      | 35-55  | About 80% insoluble   |
| Lipids        | 20-30  | Fats 50%, choline 46%, sterols 20, inositol, 22 glycerol 22, waxes 16, ethanol amine 8, phospholipids 2-7, serine 0.7 |
| Carbohydrate  | THE RESERVE OF THE PARTY OF THE | Starch, sugar phosphate (3-7%)  |
| Chlorophyll   | 9  | Chlorophyll a 75% Chlorophyll b 25%   |
| Carotenoids   | dannid To notice   | Xanthophyll 75%<br>Carotene 25%   |
| Nucleic acids |  | Major   |
| RNA           | . 2-3  |   |
| DNA           | 0.02-0.01  |   |

If aldehyde is linked, this molecule becomes chlorophyll b

H-C=0

D III

Fig. 9.3B Chlorophyll c.

Fig. 9.3C Phytol chain of chlorophyll.

situated around an atom of magnesium and a long tail constituted by a hydraulic chain (phytol chain) (Fig. 9.3C).

Carotenoid pigments are carotenes and xanthophylls. They are masked by the green colour of chlorophyll. In autumn, they can be seen because of the decrease in the amount of chlorophyll. Carotenoid pigments are related to vitamin A. In carotenes, there is a short

chain of unsaturated hydrocarbons making them completely hydrophobic. Xanthophylls,

on the other hand, possess several hydroxyl groups.

The average percentage of RNA is 3 to 4% of the dry weight of plastids. In Chlamydomonos, bodies giving Feulgen reaction typical of DNA have been, however, found within the chloroplast. If these bodies are treated with the enzyme DNAse, they disappear, showing thereby that they are DNA. The DNA in the chloroplast of Chl.:mydomonos and other chloroplasts is connected with a special non-chromosomal genetic system (cytoplasmic heredity).

Some cytochromes, vitamins K and E, and metallic atoms such as Fe, Cu, Mn and Zn have

also been found within chloroplasts.

#### Structure

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The chloroplast is covered by two membranes, each being 40 to 60 Å in thickness (Figs. 9.4 and 9.5). The space between these membranes is 25 to 75 Å. Inside, there is a proteinaceous matrix called the stroma which contains various particles and molecules such as a 175 Å diameter chloroplast ribosome, proteinaceous stroma centre, starch grains, pyrenoids (in lower plants), osmiophillic globuli, phytoferritin in some cases, fine fibrils of DNA different from nuclear DNA, etc. Osmiophillic globuli are usually covered by lamellar membranes of stroma. These granules, called plastoglobuli, contain various lipid materials. Chlorophyll or carotenoid pigments never occur in them.

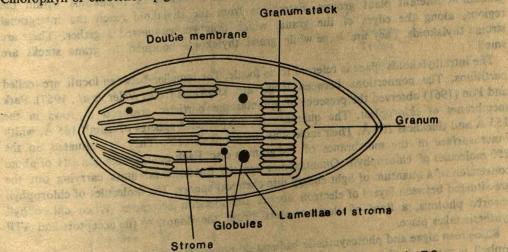


Fig. 9.4 Submicroscopic structure (diagrammatic) of chloroplast in T.S. (Redrawn from de Robertis et al., Cell Biology, 1970.)

In the stroma, there is present a lipoprotein-membrane system which contains chlorophyll. It is the site of light reactions and electron transport system which operates during photosynthesis. It consists of flattened sacs called lamellae or thylakoids. In many algae, these lamellae are arranged in a parallel fashion throughout the length of the chloroplast.

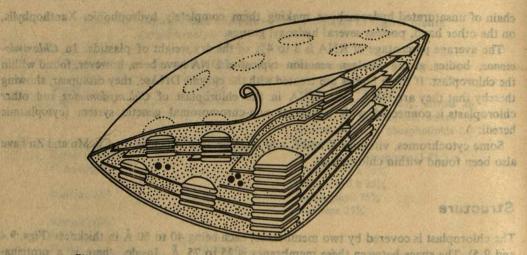


Fig. 9.5 Three-dimensional structure of chloroplast (diagrammatic) in a higher plant. (Redrawn from Wilson and Morrison, Cytology, 1966.)

In higher plants, however, the structure comprises grana that are joined by membranes. Each granum is formed by discs of thylakoids. There are about 20 to 100 grana in each chloroplast. There is, however, variation in the arrangement of thylakoids within the granum in different plant groups. Extensions from the thylakoid reach the intergranal regions, along the edges of the grana, which thus get connected together. These are stroma thylakoids. They are large while grana thylakoids confined to grana stacks are small.

The intrathylakoids space is referred to as locule. The lamellae between loculi are called partitions. The connections between loculi are known as frets (Weier et al., 1967). Park and Pon (1961) observed the presence of quantasome particles arranged in rows in the membranes of chloroplast. The quantasomes are flattened spheres of length 185 Å, width 155 Å and thickness 100 Å. Their molecular weight is  $2 \times 10^6$ . They are situated on the inner surface of the membranes of the granum disc. Each quantasome consists of about 250 molecules of chlorophyll. Quantasomes are the fundamental units carrying out the conversion of a quantum of light energy into chemical energy. The molecules of chlorophyll are situated between layers of electron donors and electron acceptors. When chlorophyll absorbs photons, a flow of electrons starts from the donors to the acceptors and ATP synthesis takes place.

Blue-green algae and photosynthetic bacteria do not contain chloroplast, although chlorophyll is present in the membrane. Because of presence of the chlorophyll, they have been regarded by some as the evolutionary ancestors of chloroplasts of higher plants. Grana are absent in *Chlamydomonos*.

Plastids are capable of RNA and protein syntheses but their ribosomes are smaller than other cytoplasmic ribosomes. It seems probable that plastids, like mitochondria possess some components formed within them and others made in the cytoplasm outside under the control of the nucleus.

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Chloroplasts are connected in the process of photosynthesis, which is divided into partial reactions as follows:

- 1. Leaf pigments absorb light energy (photons) (Fig. 9.6) and transfer it to ADP so that the latter is transformed into ATP (photosynthetic phosphorylation) (Arnon, 1955).
- Photolysis of water takes place (→ 2H<sup>+</sup>+O<sup>2-</sup>) and there is transfer of hydrogen to the system (TPN → TPNH) by ferredoxin (Tagawa et al., 1963). O<sub>2</sub> is liberated. This process is called Hill reaction. It takes place in the presence of light and a hydrogen

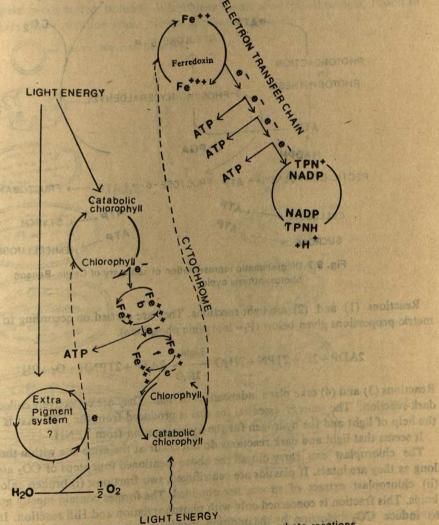


Fig. 9.6 Electron transport (diagrammatic) in primary photo-reactions in photosynthesis.

acceptor is required. In vitro, the hydrogen acceptor can be replaced by ferricyanide or quinone. DPN and TPN can also be hydrogenated.

3. CO<sub>2</sub> from the air is transferred to a C<sub>6</sub> sugar (ribulose 1-5-diphosphate) and the resulting C<sub>6</sub> compound is cleavaged into two molecules of phosphoglyceric acid (Calvin, 1962).

4. Phosphoglyceric acid is hydrogenated by the action of TPNH to phosphoglyceral-dehyde (C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>P), which is a triose. This becomes polymerised to a hexose (CO<sub>2</sub> assimilation) and regenerates ribulose (Calvin-Benson cycle) (Fig. 9.7).

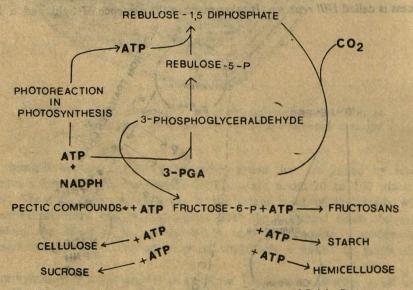


Fig. 9.7 Diagrammatic representation of summary of Calvin-Benson photosynthesis cycle.

Reactions (1) and (2) are light reactions. They are carried on according to the stoichiometric proportions given below (P<sub>i</sub>=inorganic phosphate).

$$2ADP + 2P_i + 2TPN + 2H_2O \xrightarrow{Light} 2ATP + 2TPNH + O_2 + 2H^+$$

Reactions (3) and (4) take place independent of light. They are collectively called Blackman's dark-reaction. The energy essential for this is produced from the ATP that is formed with the help of light and the hydrogen for the hydrogenation from TPNH.

It seems that light and dark reactions do not occur at the same site within the chloroplast. The chloroplast can carry out all the above mentioned four steps of CO<sub>2</sub> assimilation as long as they are intact. If plastids are centrifuged, two fractions: (i) broken chloroplasts and (ii) chloroplast extract of stroma are obtained. The former contain mostly granum thylakoids. This fraction is concerned only with phosphorylation and Hill reaction. It is possible to induce CO<sub>2</sub> reduction by the addition of chloroplast extract which contains DPN. This can be taken as a proof of the localisation of the hydrogen-transfer system in the stroma.

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Proplastids are covered by a double membrane. The inner membrane grows in the presence of light and gives off vesicles that arrange in such a way as to form large discs. There are inbuilt stacks of closely-packed lamellar sacs or thylakoids in the regions of the grana (Fig. 9.8). In a fully developed chloroplast, some compartments of grana are seen as connected by intergranal membranes or tubules. Lack of light affects this developmental process very much. When plants are grown under a light of low intensity, there is aggregation of vesicles which are formed in the proplastid. In this manner, one or several prolamellar bodies are formed. Sometimes, a crystalline pattern is formed by the vesicles. This pattern consists of regularly-connected tubules. When these plants are exposed to light, fusion of vesicles into layers may occur and grana may be developed again.

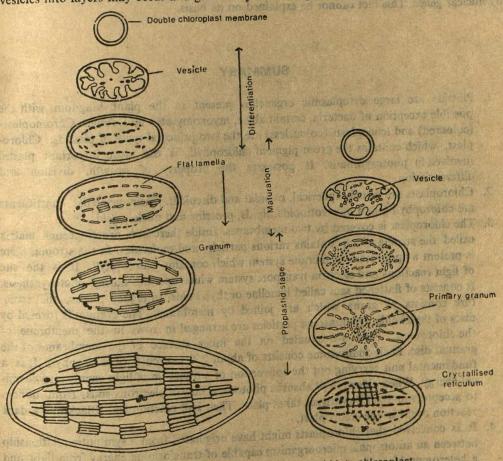


Fig. 9.8 Stages in development of proplastid into chloroplastic states of a

Like mitochondria, chloroplasts are constituted of two membranes, each having different functions. The inner membrane, which contains the photosynthetic- and electron-transport systems, forms the grana. The intergranal tubules remind one of mitochondrial crests.

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The inner membrane and connected structures are controlled by genetic factors present in the nucleus and chloroplast (DNA) and also by external factors such as light, plant hormones, metabolic inhibitors and minerals.

#### Symbiotic Origin

Chloroplasts possess the property of growth, division and differentiation. Since they contain DNA, ribosomal RNA and mRNA, they are capable of conducting protein synthesis. It is suggested that chloroplasts might have originated from a symbiotic relationship between an autotrophic microorganism capable of transforming energy from light and a heteromorphic cell. There is, however, one drawback in the hypothesis. The electron-transport system in chloroplasts and the enzymes necessary for producing photosynthetic pigments are controlled by nuclear genes. This fact cannot be explained on its basis.

#### SUMMARY

- 1. Plastids are large cytoplasmic organelles present in the plant kingdom with the possible exception of bacteria, certain algae, myoxomycetes and fungi. Chromoplasts (coloured) and leucoplasts (colourless) are the two principal types of plastids. Chloroplast, which contains the green pigment chlorophyll, is the most important plastid involved in photosynthesis. It possesses the property of growth, division and differentiation.
- 2. Chloroplasts are usually spherical, ovoidal and discoidal. Their principal constituents are chlorophyll a and b, carotinoids, lipids, proteins and nucleic acids.
- 3. The chloroplast is covered by two membranes. Inside there is a proteinaceous matrix called the stroma which contains various particles and molecules. In the stroma, there is present a lipoprotein membrane system which contains chlorophyll. It is the site of light reactions and electron transport system which operates during photosynthesis. It consists of flattened sacs called lamellae or thylakoids. In higher plants, the structure consists of grana which are joined by membranes. The granum is formed by discs of thylakoids. Quantasome particles are arranged in rows in the membrane of the chloroplast. They are situated on the inner surface of the membranes of the granum disc. Each quantasome consists of about 250 molecules of chlorophyll. It is a fundamental unit carrying out the conversion of a quantum of light energy into chemical energy. When chlorophyll absorbs photons, a flow of electrons starts from donors to acceptors and ATP synthesis takes place. Then Hill reaction and Blackman's dark reaction occur in the chloroplast.
- 4. It is conceived that chloroplasts might have originated from a symbiotic relationship between an autotrophic microorganism capable of transforming energy from light and a heteromorphic cell.

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Singh, S.P. and B.S. Tomar (1971), An Introduction to Cytology, 3rd edn., Rastogi Publications, Meerut. or there may be a coroniaging rating parter (Fig. 10.1), although some express double about

# 10. Vacuoles and Other Organelles Amenad, I. (1967) "The Oremination and

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There is considerable controversy as to whether a vacuole can be treated as a cell organelle. A vacuole is an aggregation of solid or liquid materials. It may be covered by a membrane or there may be a cytoplasmic lining inside (Fig. 10.1), although some express doubts about this. Some vacuoles seem to represent vesicles formed by the endoplasmic reticulum. They have fibrillar extensions which appear similar to the reticular component.

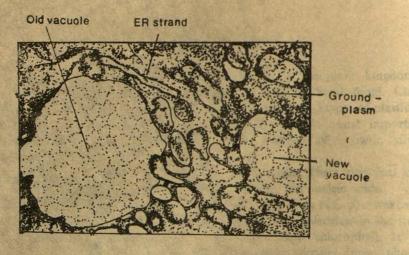


Fig. 10.1 Development of vacuole (diagrammatic). (Redrawn from Frey-Wyssling and Muhlethaler, Ultrastructural Plant Cytology, 1965.)

In fully developed plant cells, there is a large central vacuole enclosed by a semipermeable membrane called the tonoplast. It was De Vries (1885) who first used the term tonoplast to mean an autonomous vacuole-producing particle. Pfeffer (1890), however, denied the presence of such an organelle. He stated that vacuoles were produced by striking hydration of certain area in the cytoplasm. The tonoplast is usually a firm covering. It is mechanically stronger than the plasmalemma. In certain cases, however, it may be similar to the external plasma membrane in its physical nature. When the tonoplast is studied under an electron microscope, it is seen as a unit membrane and appears like the plasmalemma. As a permeability barrier, the tonoplast is usually regarded stronger than the plasmalemma.

When plant cells expand, the vacuoles become very large. This is regarded as due to lack of sufficient nitrogenous nutrient materials. It is a fact that plants are unable to produce enough protein to fill all its expanded cells with cytoplasm. Hence, the watery cell sap of the central vacuole fills the available space.

The vacuole functions as an osmometer because it is lined by the cytoplasmic layer and both the tonoplast and plasmalemma are semipermeable membranes. The molar concentration of the cell sap sets in the process of osmosis and the cell acquires the necessary firmness or turgidity. Vacuoles secrete assimilatory products such as sugars and proteins and act as storage for these materials which are used in the cell metabolism whenever required. Sugars are present in the vacuoles as solutes while proteins are present in solid state, e.g. aleurone grains in the endosperm.

Marinos (1963) has shown that vacuoles originate from the Golgi complex. This means that the tonoplast is a Golgi membrane, i.e. homologous to the plasmalemma.

## Cilia, Flagella and Basal Bodies

Cilia (Fig. 10.2) and flagella (Fig. 10.3) are extensions of the cell surface. They originate from cytoplasm and are bounded by the cell membrane. Many protozoans, flat worms and

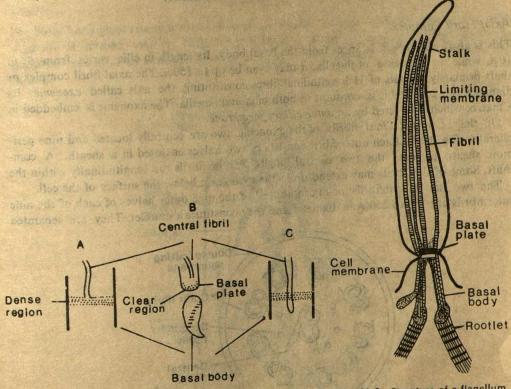


Fig. 10.3 Structure of a flagellum. Fig. 10.2 Diagram showing relation between cilium and basal

A-From mammal, B-From mollusc and amphibian.

C-From Tetrahymena.

plant cells (e.g. gametes of algae, aquatic fungi, and ferns) possess these structures. They are the chief organelles of locomotion in protozoa.

There is no clearcut morphological or physiological distinction between cilia and flagella. Usually, when the extension is shorter than the cell, the term 'cilium' is used and when it is longer, it is called 'flagellum'. Cilia are usually larger in number than the flagella. The length of cilia generally varies between 3 and  $10 \mu$ . Flagella may be even up to  $150 \mu$  in length. Usually there are one or two flagella in a cell.

#### Structure

The cilium possesses the limiting membrane, axial fibril complex (shaft), basal body and in some cells rootlets.

#### Limiting Membrane

It is a double membrane which encloses the entire axial complex. It is continuous with the plasma membrane of the cell. The two membranes are separated from each other by a space of 90 Å. The inner membrane is about 40 Å in thickness.

Chia, Fiagella and Bussi Rodies

#### Axial Fibril Complex

This is also called *shaft*. It arises from the basal body. Its length in cilia varies from 5 to  $10 \,\mu$ , while in the case of flagella, it may even be up to  $150 \,\mu$ . The axial fibril complex or shaft primarily consists of 11 longitudinal fibres constituting the axis called *exoneme*. Its diameter is 8.21  $\mu$  and it is constant in both cilia and flagella. The axoneme is embedded in a matrix which is covered by an *outer ciliary membrane*.

Of the 11 longitudinal fibrils of the axoneme, two are centrally located and nine peripherally (Fig. 10.4). Each outer fibril consists of two halves enclosed in a sheath. A common sheath encloses the two central fibrils. All the fibrils run longitudinally within the shaft. Some of the fibrils may extend up to the cytoplasm below the surface of the cell.

The two halves (subfibrils or microtubules) of the nine outer halves of each of the nine outer fibrils are 180 to 250 Å in diameter and they constitute a doublet. They are separated

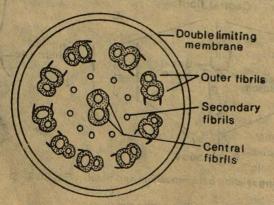


Fig. 10.4 T.S. of a flagellum (diagrammatic).

from each other by a wall of 45 Å in thickness. These doublet tubules are connected to each other and to the central tubules. There may be additional fibres in the distal part of the flagellum between the outer and central fibrils. There are nine of these secondary fibrils. They are smaller in size, the diameter of each being about 30 Å.

#### Basal Body

The cilium or flagellum is connected with a particle situated in a clear cytoplasmic layer just below the plasma membrane (Figs. 10.2 to 10.4). This particle is called the basal body. In cilia, numerous basal bodies are arranged in a parallel row beneath the cell membrane. The structure of the basal body of the cilium is different from that of the flagellum. In case of the former, the nine outer fibrils are composed of three units (triplets) or subfibrils while in the latter, there are doublets. Two of these triplets form a doublet in the flagellum. The third unit ends between the basal body proper and the flagellum. The region which is attached to the basal body is situated near the cell surface within the external part of the organelle. It is called the zone of transition.

#### Rootlet

The basal body gives rise to a thread-like structure that extends into the cytoplasm. This structure is termed 'rootlet' (Figs. 10.3, 10.5 and 10.6). It is usually found in ciliated epithelial cells of lower animals. It seems as if the rootlet anchors the basal body to the cilium.

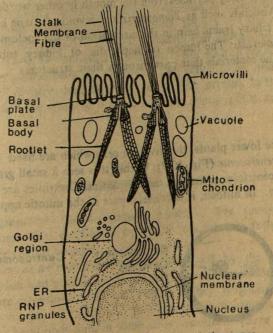


Diagram of a flagellum and the inner Fig. 10.5 contents of a cell-

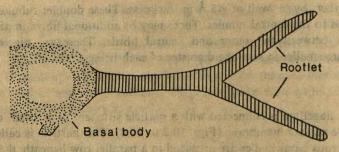


Fig. 10.6 Basal body with rootlet.

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#### **Functions**

Cilia and flagella are involved in the following functions:

- 1. Locomotion: This is achieved by means of cilia or flagella in ciliate and flagellate protozoa respectively, Ctenophores, certain Platyhelminthes and Nemertea, ciliated larvae of Annelida, Mollusca, Echinodermata, etc.
- 2. Feeding: Several sluggish animals such as Amphioxus and Herolmania, are ciliary filter feeders. These animals trap fine particles of food brought by ciliary current.
- 3. Respiration: The exchange of gases is facilitated because of the continuous movement of water caused by the cilia.
- 4. Cleansing: The cilia on the tentacles and oral region in case of many sea anemones and corals sweep away particles from the mouth in other words, they cleanse it.
- 5. Circulation: Annelids such as Tomopteris are devoid of a true blood vascular system. In them, the ciliary tracts help in the circulation of the coelomic fluid.
- 6. Passage of materials: The lumen of nephridia or kidney tubules is lined by cilia which assist the materials that pass through these structures.

#### Centrosome

In animals and some lower plants, the dividing cells which are fixed and stained, show a clear zone called *centrosome* (Fig. 10.7). Within it is seen a small granule termed the 'centriole'. One or two centrioles constitute the cell centre. Centrioles are not observed except during cell division. In mitosis, it becomes a part of the mitotic apparatus. Centrioles are

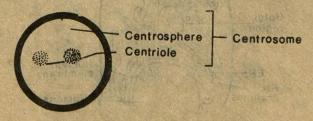


Fig. 10.7 Centrosome in interphase.

made up of hollow cylinders (Fig. 10.8) or pairs of cylinders, which are actually bundles of small rods or fibres. They are arranged parallel to the axis. The bundles are grouped in seven sets of three each. A cilium has a bundle of two fibres. The length of the centriole is about  $150 \mu$ . The interior of the cylinder is less dense.

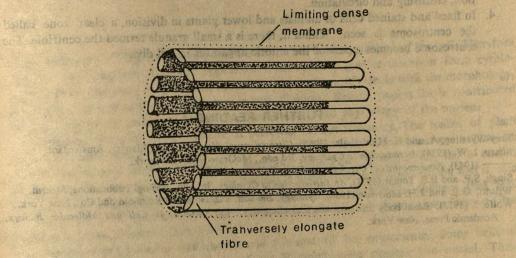


Fig. 10.8 Structure of the centriole.

The clear zone seen around the centriole during cell division is called the 'centrosphere'. It, together with the centriole, constitutes the centrosome. During cell division, the centriole divides and forms two centrioles. They separate from each other and go to opposite poles of the spindle (see Fig. 13.4). Generally, the centriole is not seen in plant cells (especially in higher plants and many animal cells). It seems that the centrosome and centriole have an important role in cell division, but, this has not yet been proved. In this connection, it should be noted that though the centriole is not present in many plant cells, they divide quite normally.

## SUMMARY

- 1. A vacuole is an aggregation of solid or liquid materials. The semi-permeable membrane which encloses this vacuole is called the tonoplast. In fully developed plant cells, there is a large central vacuole.
- The vacuole functions as an osmometer because it is lined by the cytoplasmic layer and both the tonoplast and plasmalemma are semipermeable membranes.
- 3. Cilia and flagella are extensions of the cell surface. They originate from cytoplasm and are bounded by the cell membrane. A cilium comprises the limiting membrane, axial fibril complex, basal body and, in some cells, rootlets. There is, however, no clearcut morphological or physiological distinction between cilia and flagella. When

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the extension is shorter than the cell the term cilium is used, and when it is longer, it is called a flagellum. These structures are connected with a particle called the basal body situated in a clear cytoplasmic layer just below its plasma membrane. Cilia and flagella are involved in several functions like locomotion, feeding, respiration, cleansing and circulation.

4. In fixed and stained cells of animals and lower plants in division, a clear zone called the centrosome is seen. Within it, there is a small granule termed the centriole. The

centrosome becomes a part of the mitotic apparatus in cell division.

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## 11. Nucleus the commence of the state of th The interplace of the state of

The nucleus is the largest organelle and the controller of the activities of the cell. Therefore it is not surprising that of the cell organelles, it has received greater attention from cytologists since its discovery by Brown in 1835. The old work mostly deals with the morphology, especially in regard to the chromosomes in cell division. Recent papers have contributed considerably to our understanding of the biochemistry and physiology of the nucleus.

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The nucleus has a complex structure. It performs various functions. The nuclei of plant and animal cells seem to have the same structure and functions. The nucleus passes through two phases, namely, interphase (which is metabolic phase) and mitotic phase, i.e. a period of division. In the former phase, the nucleus is under a state of non-division and is said to be in the resting stage. In the latter phase, the nucleus undergoes changes that lead to the division of chromosomes and consequently two daughter nuclei are formed (Red blood corpuscles, however, lack nuclei).

Generally, a cell possesses one nucleus and it is said to be uninucleate. Some cells of liver or tapetal cells of some members of the Euphorbiaceae possess two nuclei. These are termed as binucleate. When cells possess many nuclei, they are called polynucleate, e.g. some cells of the endosperm of seed.

# Interphase Nucleus

The interphase nucleus functions between mitoses in dividing and growing cells. The synthesis of RNA and various cytoplasmic components takes place during the interphase mainly in the  $G_1$  phase and to a lesser degree in the S phase during DNA synthesis before mitosis as appropriate and the assent of the secretary of support

The shape of the nucleus is usually spherical but it may be fusiform, elliptical, flattened or irregular. Sometimes it depends on the function. In young cells, the nucleus is generally spherical and situated in the centre of the cell, but in differentiated cells, it may be displaced from the central position and may assume an irregular shape. The nuclear size has a constant relation to the cytoplasmic volume which is expressed numerically as the nucleo-Allo our Eave one The state of the second s

$$NP = \frac{Vn}{Vc - Vn}$$

where Vn = nuclear volume and the same decay and the same and the s Vc = volume of cytoplasm with a discharge and a second as the second as and do pores about 800 A in discrete. At the ports, the cuber and anner ment the man and but these together at their retires, trained (1966-7.2) manufact the attracture of percent details It seems that the lack of maintenance of NP ratio acts as a stimulus to cell division.

The interphase nucleus (Fig. 11.1) possesses (i) a nuclear envelope or karyotheca, (ii) nucleoplasm, a nonstaining or slightly chromophilic mass, (iii) chromosomes, which are dispersed in nucleoplasm, (iv) chromocentres, and (v) a nucleolus, which is a basophilic body.

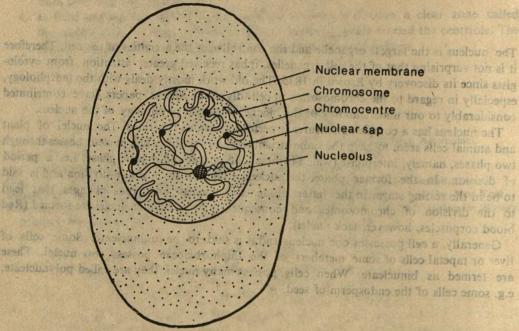


Fig. 11.1 Schematic representation of interphase nucleus showing example of chromosome parts in contact with nuclear boundary and nucleolus.

If the nucleus is stained with uranyl acetate, both the nucleic acids are stained but DNA comes out most conspicuously. The chromatin channels which lead to the nuclear pores can also be seen. The nucleolus is stained densely while the associated chromatin surrounding it stains more densely. The nucleoplasm stains lightly and shows fine granules.

# spherical and situated in the centre of the cell, but in differentiated cells, it may be displaced from the central position and may assume an irregular shape. The nuclea artificially as a seconstant relation to the cytoplasmic volume which is expressed numerically as a se nucleo-

#### Nuclear Envelope

When the nucleus is studied under an electron microscope, the nuclear envelope stands out as the most characteristic feature. The nuclear envelope which surrounds the nucleus is a double-membraned structure (Fig. 6.4). Both the unit membranes are 80 Å thick. They are separated by a space (perinuclear space) which is about 150 Å broad. They are perforated by pores about 800 Å in diameter. At the pores, the outer and inner membranes are fused together at their edges. Franke (1966-74) has studied the structure of pores in detail in the nuclear envelope isolated from onion cells.

The border of the pore is an annulus exhibiting an eight-fold symmetry. Pores may occupy about 8% or more of the area of the nuclear envelope. This means that in the case of a small nucleus (e.g. yeast nucleus, with a diameter of  $2\mu$ ), there are about 200 pores or in higher plants like onion (with a nucleus whose diameter is about  $8 \mu$ ) there are about 3000 pores per nucleus. Franke et al. (1966-70) have proposed a model according to which the annular material present within the nuclear pores extends largely beyond the margins of pores. Within the pores, one to three central granules are seen which are likely to be ribonucleoprotein particles passing through the pores. Chemical analysis of isolated nuclear membranes shows that they are made up of proteins (59-75%) and lipids (17-35%).

In many lower plants, particularly in fungi, the nuclear envelope remains intact during the division of the nucleus and at the time of cytokinesis it divides and is partitioned equally to the daughter nuclei (Hawker, 1965). In higher plants, however, the nuclear envelope becomes ruptured at the end of prophase and disintegrates and thus becoming indistinguishable from the endoplasmic reticulum which comes close to the chromosomes. During telophase, the pieces of the broken nuclear envelope join up in some way and subsequently become continuous resulting in the formation of a new nuclear envelope around the chromosomes (Porter and Machado, 1960; Lafontaine and Chouinard, 1963). The outer membrane of the nuclear envelope is continuous with the endoplosmic reticulum (Fig. 6.4). This fact points out that they are, in fact, a single system of membranes (Marinos, 1960). Jensen (1964) states that the nuclear envelope of fusion nuclei in the enbryo sac is derived equally from the two polar nuclei which form the polar nucleus.

The endoplasmic reticulum is continuous through the plasmodessmata from one cell to the next. Therefore, it is very likely that the perinuclear spaces of nuclei in adjacent cells are in direct continuity. Hence, there would be transport of substances through the lumen of the endoplasmic reticulum from one nucleus to another without coming into contact with any other components of the cytoplasm. The presence of many electron-transport enzymes has been reported within the nuclear envelope of liver cells and their concentrations are comparable to those in mitochondria. It, therefore, appears that the nuclear envelope is probably involved in the oxidative phosphorylation and electron transport. Structures which probably represent the chromatin of the nucleolar organiser can be

Nuclear Sap: 1) the grant of the nucleolus by treatment with a detergent (Liqual result) The nuclear sap (nucleoplasm or karyolymph) is present inside the nuclear envelope and the other nuclear components are scattered in it. It appears to be a somewhat granular and homogeneous fluid. Of the substances which are dissolved or suspended in it, are also those that are in transit from the nucleus to the cytoplasm. Ribonucleoprotein-like granules and fine filaments of unknown nature are visible.

As regards the chemical nature of the nuclear sap, little information is available. Ultracentrifugation studies show that it is essentially protein in character. Some RNA occurs in it and therefore positive cytochemical reactions for glycoproteins are obtained. Many hydrolytic enzymes (e.g. ribonuclease, diapeptidase and alkaline phosphatase) are present in the nucleus. They may act as specific constituents of the nucleoplasm (Brachet, 1957).

again at telophuse. Electron microscope study indicates that the nucleolus in late prophase becomes more loosely organised until the fibrils of 100 Å diameter merge with the surround-

## Nucleolus a remmy bloba sio no punteletze sulcome na se mod sult lo inbrod sult

The nucleolus was first observed by Fontana in 1874. It is a relatively large prominent body present in the nucleus. It is usually spherical in shape.

Interphase nuclei may possess one or more nucleoli depending on the species. The number of nucleoli may remain constant or it may vary. On the basis of present evidence, it seems that nucleoli in the somatic and reproductive cells of many plants and animals are formed during the mitotic telophase in association with the nucleolar organiser of specific chromosomes of the complement which are called nucleolar chromosomes. A large number of diploid plants possess a single pair of nucleolar chromosomes. In this case, the nucleolus which is formed in association with each of the two homologous chromosomes frequently fuses with the other and thus a single fusion nucleolus is formed in interphase. In some diploid species, more than one pair of chromosomes, however, are involved in the nuclear organisation.

able of the enderglasmic reflection which comes close to the characteristic forms

#### Structure is the smalle being making as the structure is the series of t

Since the nucleolus does not possess a membrane, it is in direct continuity with the rest of the nucleus. Electron microscope studies (Day, 1968; Bernhard and Granboulan, 1968; Bush and Sematana, 1970) indicate that the nucleolus is composed of a network of fibres about 20 Å in diameter. The fibres are made of fibrils about 20 Å in diameter. As the fibres stain for protein and are destroyed by RNase and not by DNase, they must be ribonucleoprotein (Hyde et al., 1965; La Cour, 1966). Granules about 150 Å in diameter are present in different parts of nucleolus. The granules probably represent compactly packed fibres. The granules which are frequently observed around the periphery of the nucleolus are the precursors of ribosomes. As they are at the periphery, the central part is virtually free of them. It consists of fibrils in an amorphous protein matrix. The fibrillar and granular regions of the nucleolus can also be observed under the light microscope (Fig. 11.2) using a special silver impregnation technique. The granular region is seen as consisting of strands of about 1000 Å thickness which were thought by Estable and Sotelo (1952) as a continuous coiled filament called nucleolonema embedded in a homogeneous matrix termed pars amorpha.

Structures which probably represent the chromatin of the nucleolar organiser can be obtained from the central part of the nucleolus by treatment with a detergent (La Cour, 1966). They appear to be analogous to the nucleoli of amphibian eggs (Callan, 1966). Electron-micrograph of Spirogyra clearly shows the association of the chromatin of the nucleolar organiser with the nucleolus (Godward and Jordan, 1965). This fact leads to the conclusion that the nucleolus is a collection of the gene products and possessing sufficient morphological structure which remains associated with the loci at which they were formed. In other words, the nucleolus is associated with DNA. It is found that the plant nucleoli contain about 10 to 20% of the nuclear DNA.

## 

In dividing cells, the nucleolus normally disappears at the end of prophase and is formed again at telophase. Electron microscope study indicates that the nucleolus in late prophase becomes more loosely organised until the fibrils of 100 Å diameter merge with the surround-

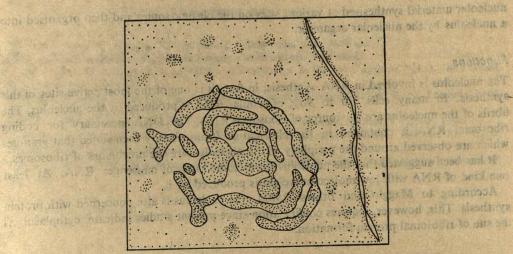


Fig. 11.2 Diagram of electron-micrograph of structure of nucleolus. Granular and fibrillar structure is seen. The central region (amorphous matrix) is more homogeneous. (Redrawn from Wilson and Morrison, Cytology, 1966).

ing nucleoplasm and there is dispersion of granules of 150 Å diameter throughout the nucleoplasm.

It has long been recognised that the formation of new nucleoli in telophase is a function of the nucleolar organiser (Fig. 11.3). Hence, initially the number of nucleoli and organisers are the same. When the newly formed nucleoli are first seen in telophase, the chromosomes is observed to be coated with a material which is fibrillar-granular in texture. This material however, disappears in late prophase. According to some cytologists, it represents the

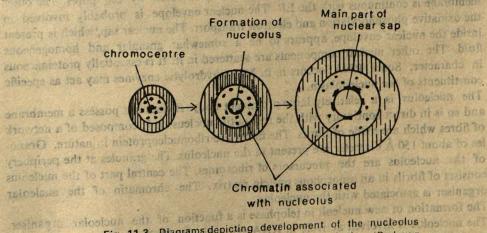


Fig. 11.3 Diagrams depicting development of the nucleolus from chromatin associated with nucleolus. (Redrawn from Wilson and Morrison, Cytology, 1966.)

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nucleolar material synthesised at various sites on the chromosomes and then organised into a nucleolus by the nucleolar organiser.

#### **Functions**

The nucleolus is involved in RNA synthesis, in fact, it is one of the most active sites of this synthesis. In many cells, 70 to 90% of cellular RNA is produced by the nucleolus. The fibrils of the nucleolus are the source of ribosomal RNA. The DNA necessary for coding ribosomal RNA is contained in the chromatin. It has already been stated that granules which are observed around the periphery of the nucleolus are the precursors of ribosomes.

It has been suggested that the nucleolus forms some kinds of messenger RNA. At least one kind of RNA with low molecular weight is produced by it.

According to Maggio and others (1963), the nucleolus is also concerned with protein synthesis. This, however, requires confirmation since present studies indicate cytoplasm as the site of ribosomal protein formation.

#### SUMMARY

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- 1. The nucleus is the largest organelle and the controller of the activities of the cell. The interphase nucleus functions between mitosis in dividing and growing cells. The synthesis of RNA and various cytoplasmic components takes place during the interphase mainly in the  $G_1$  phase and to a lesser degree in the S phase during DNA synthesis before mitosis.
- 2. The interphase nucleus comprises a nuclear envelope, nucleoplasm, chromosomes (dispersed in nucleoplasm), chromocentres and nucleolus. The nuclear envelope is a double membrane. The unit membranes are perforated by the pores. The outer membrane is continuous with the ER. The nuclear envelope is probably involved in the oxidative phosphorylation and electron transport. The nuclear sap, which is present inside the nuclear envelope, appears to be a somewhat granular and homogeneous fluid. The other nuclear components are scattered in it. It is essentially proteinaceous in character. Some RNA occurs in it. Many hydrolytic enzymes may act as specific constituents of the nucleoplasm.
- 3. The nucleolus is a relatively large prominent body. It does not possess a membrane and so is in direct continuity with the rest of the nucleus. It is composed of a network of fibres which are made of fibrils. The fibres are ribonucleoprotein in nature. Granules of about 150 Å diameter are present in the nucleolus. The granules at the periphery of the nucleolus are the precursors of ribosomes. The central part of the nucleolus consists of fibrils in an amorphous protein matrix. The chromatin of the nucleolar organiser is associated with the nucleolus.
- 4. The formation of new nucleoli in telophase is a function of the nucleolar organiser. The nucleolus is involved actively in RNA synthesis. Its fibrils are the source of ribosomal RNA. It may form some kind of messenger RNA.

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# 12. Chromosomes

In 1875, Strasburger discovered thread-like structures which appeared during the division of the nucleus. They were named 'chromosomes' by Waldeyer because of their affinity towards basic dyes.

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In all eukaryotes, the nucleus possesses a definite number of chromosomes of individual sizes and shapes. Although they remain invisible in the nucleus at the metabolic stage, they become visible at the beginning of mitosis, i.e. at prophase. For each species, there is a certain definite number of chromosomes characteristic of it. This number becomes halved when gametes are formed in the process of meiosis (reduction division).

#### Morphology

Chromosomes undergo major morphological changes during cell division. The details of their morphology, however, vary from cell to cell. Each chromosome is an undivided entity and it remains in the cell throughout the life of the latter. Earlier workers thought that chromosomes were formed during interphase either due to the coalescence of individual particles or they were joined together to form a continuous chain. These ideas have now been completely rejected.

Electron microscope study of chromosomes has not yet yielded data on the basis of which their structure could be described in detail, but present information indicates that a chromosome consists of chromonemata (Fig. 12.1) which comprise many microfibrils measuring about 60 Å units. There is no unanimity about the total number of these microfibrils, but it is probable that the number is above 64. The present data indicate that each chromosome is composed of at least two bundles of microfibrils. Parts of some chromosomes are seen as highly condensed areas in the interphase nucleus. These are called *chromocentres* (Fig. 11.1) or *heterochromatic segments* (prochromosomes) and are generally regarded as representing tightly-coiled chromosome regions.

In 1964, Cohn used the term 'chromatin' to describe the DNA-containing structure of the nucleus. Certain areas of chromatin stain darker than others. Such regions are called heterochromatin. Heterochromatic segments are more condensed than the others. Such a difference in staining is called heteropycnosis which is characteristic of heterochromatin. When there is overcondensation, heteropycnosis is positive (staining more strong), and when there is undercondensation, it is negative (staining weakly). Chromosomes that remain in the condensed state are known as heterochromosomes (e.g. sex chromosomes of insects). Those regions of chromosomes which do not exhibit heteropycnosis are called euchromatic regions

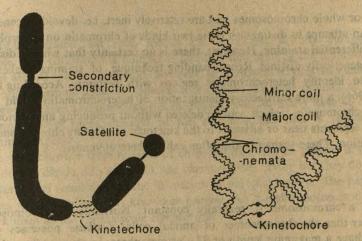


Diagram of a submetacentric chromosome. On the left is its external form and on the right its internal structure showing two chromonemata and major and minor coils.

and the part comprising them, the euchromatin. The non-condensed chromosomes which extend during interphase are called euchromosomes.

According to some authors, the heterochromatic regions have a higher RNA content as compared to the euchromatic regions. In some cases, large areas of the nucleus stain intensely with basic fuchsin. A chromocentre may comprise heterochromatic regions of several chromosomes or in certain cases all the chromosomes of the nucleus, e.g. gland cells of Drosophila and Sciara. In these animals, it has been observed that the rate of RNA synthesis in the heterochromatic regions is different from that in the euchromatic regions.

Heterochromatin usually corresponds to the segment of chromosomes which are persistently heteropycnotic under any condition. The clear segments, which have low concentrations of nucleic acid, show deficiencies during cell division. The consistency of distribution of such clear segments make them useful for characterisation of each chromosome of the group. DNA is more labile in heterochromatin. Heterochromatin regions correspond to those regions where the chromonema has a differential degree of coiling. The chromonema is continuous from the euchromatic to the heterochromatic region.

The position of the chromomeres is relatively constant for a given chromosome. According to some authors, chromomeres represent condensation of nucleoprotein material, while others maintain that they are regions in which there is superimposition of coils. Electron microscope study gives some support to the latter contention. Previously it was held that chromosomes are an amorphous matrix between the pellicle surrounding the chromosome and the coils of chromonema. Recent electron microscope observations have, however, disproved this concept. The present work based on micromanipulation and treatment with uncoiling agents such as KCN has shown that the chromosomes are simply regions of tight coiling.

Genetically, euchromatin is regarded as the part of the chromosome which can be demonstrated to contain the genetic material (genes), whereas heterochromatin represents

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segments of or whole chromosomes that are relatively inert, i.e. devoid of genes. Cytologists have made an attempt to distinguish these two kinds of chromatin on a morphological basis employing differential staining. However, there is no certainty that what is distinct cytologically is also genetically distinct. Recent banding technique of staining makes it easy for cytologists to identify heterochromatic regions with certainty. According to Wilson and Morrison (1966), a more satisfactory identification of heterochromatin would be that segment of a chromosome which can be deleted without producing any obvious phenotypic change. The segments near or adjacent to the kinetochore of many chromosomes are covered by this definition. These segments also often exhibit heteropycnosis.

#### Size

The size of a chromosome is generally constant. Normally, the chromosomes of plant cells are larger than the chromosomes of animal cells. *Trillium* possesses chromosomes which may have a maximum length of  $32 \mu$  at metaphase. In *Drosophila melanogaster*, the total length of all the somatic chromosomes is  $7.5 \mu$  while that of the polytenic chromosomes (salivary gland chromosomes) is  $2.000 \mu$ .

#### Shape

Depending upon the position of the kinetochore, chromosomes are classified into four types (Fig. 12.2):

- 1. Acrocentric or subtelocentric Rodlike chromosomes with one arm very small and the other very long. The kinetochore is subterminal.
- 2. Submetacentric Chromosomes with unequal arms and resembling the letter J.
- 3. Metacentric Chromosomes with equal arms resembling the letter V.
- 4. Telocentric Location of kinetochore at the end of the chromosome. Telocentric chromosomes are unstable. Different types of chromosomes are constant for species and even a genus. Hence, the shape of chromosomes is of great value in the study of karyotype. It emphasises the importance of their individualisation.

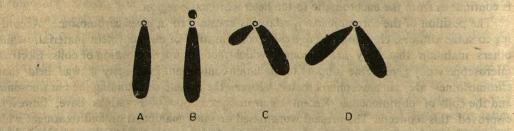


Fig. 12.2 Four types of chromosomes on the basis of the position of the kinetochore. A—Telocentric. B—Acrocentric. C—Submetacentric. D—Metacentric.

#### Number

It has already been stated that each species has a certain definite number of chromosomes. Hence, the chromosome number is useful in determining the taxonomic position of the plant and animal species. It should, however, be remembered that the number of chromosomes does not determine a species. Several species have the same number of chromosomes [e.g. man, privet hedges and black mollies (a tropical fish) normally have 46 chromosomes]. It is the composition of genes situated in the chromosomes that is responsible for the differences among species.

In some organisms, a large number of chromosomes may be present in certain types of cells which do not divide. In such cases, the chromosomes divide but they do not separate into daughter nuclei resulting into the formation of polyploid nuclei. The haploid (n) set of chromosomes which is inherited is known as genome. All the chromosomes in a nucleus are collectively known as the chromosome complement.

## (i) Primary Constriction (Kinetochore)

In most chromosomes, the kinetochore or centromere (Fig. 12.1) is the primary differentiation region that seems to be responsible for the movement of chromosomes at meiosis (Dnyansagar, 1961). The spindle fibres presumably attach at this point but we do not yet know the exact nature of the attachment. The kinetochore may have additional metabolic significance because its division takes place at a different time after replication of other regions of the chromosome (Lime-de-Faria, 1958). Its position in the primary constriction at a certain point along the chromosome (terminal, subterminal, median, etc.) is apparently fixed during evolution. Under the light microscope, it is seen as a dense granule surrounded by a clear unstained region. Since this is somewhat similar to the centriole, it was suggested that there might be some relationship between the two (Schnader, 1936; Pollister and Pollister, 1943). However, electron microscope studies do not support this suggestion (Gall, 1961). The kinetochore is seen as a dense body that may be conical in profile without any significant internal structure. Its relationship to the remainder of the chromosome is not yet clear. Figure 12.3 indicates the kinetochore organisation suggested by

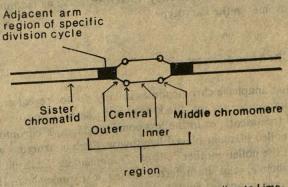


Fig. 12.3 Kinetochore organisation according to Limede-Faria.

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Lime-de-Faria. The chromosomes of some species, e.g. Hemiptera, possess the properties of kinetochores along the entire length and hence are said to have a diffuse kinetochore.

## (ii) Secondary Constriction (Fig. 12.1)

This is usually seen as a Feulgen-negative, weakly-staining gap in which a fine chromatin strand connects the main chromosomal mass. Secondary constrictions are of two kinds. Those attached to the nucleolus are called nucleolar organisers and these that are not simple are referred to as secondary constrictions. The secondary constrictions are regarded as negatively heterochromatic regions that correspond to heterochromatic or other morphologically distinguishable masses of the interphase (La Cour, 1951). They are, of course, visible only when the chromosomes become condensed. Resende (1945) called them olistherozones. Secondary constrictions are useful in the identification of particular chromosomes in the karyotype.

#### **Telomeres**

Chromosomes terminate at either ends in a fine unique structure called *telomere*. Telomeres exhibit certain specific properties. If a chromosome breaks into segments, the broken segments fuse again, but the uniting pieces do not fuse with the telomeres. It appears as if telomeres have a polarity which prevents other segments from joining with them. It is observed that in meiotic prophase, telomeres are frequently attached to the centriole and they seem to migrate to the nuclear membrane resulting in what is known as the bouquet stage.

#### Satellites

In some chromosomes (Fig. 12.4) the secondary constriction marks the formation of a round or elongated body called the satellite (Fig. 12.4). This body is separated from the remaining chromosome by a thin chromatin filament. The diameter of the satellite may be the same as that of the chromosome or smaller. A chromosome possessing a satellite is called a SAT-chromosome. The satellite and filament are always constant in form and size for each particular chromosome. They, therefore, serve as landmarks in the identification of such chromosomes in the karyotype.

#### Structure of Chromosomes

During metaphase and anaphase chromosomes usually do not reveal any internal structure under the light microscope except the kinetochore, satellite and secondary constrictions. However, by special treatments, or in the less compact stages, a chromosome can be seen as consisting of a coiled filament placed lengthwise. Such a structure was first reported by Baranetzky (1880) in the pollen mother cells of *Tradescantia*. Each linear subunit of the chromosome, in principle, can be referred to as a *chromonema*. How many filaments constitute a chromonema is not yet fully known. It may comprise two or more filaments during telophase. The number of fibres may also vary in different tissues of the same

organism. According to Kaufmann (1948) and Schrader (1948), a chromosome may possess a single fibre at one stage and become two- or four-stranded at another stage during development. Two or more chromosomal fibres are coiled together. The coiling forms two types of spirals—a paranemic coil and a plectonemic coil (Fig. 12.5). In the paranemic coil, subunits are freely separable while in the plectonemic coil, they are not easily separable since the fibres are interwined. If these fibres are drawn out, they turn into what is known as a relational coil.

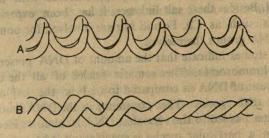


Fig. 12.5 The two types of coils formed by two or more chromosomal fibres. A-paranemic coiling, B-Plectonemic coiling.

The degree of coiling in mitotic and meiotic chromosomes (Fig. 12.6) is variable. It depends upon the length acquired by the chromosomes during cell division. A meiotic chromosome is considered to be consisting of two distinct coils (Fig. 12.1), a major coil which is about 10-30 gyres, and a minor coil, which lies perpendicular to the major coil and consists of many gyres. In the somatic chromosome, a helical structure similar to the major coil can be seen. This is the standard or somatic coil.

Radiation damage studies indicate that each chromosome contains at least four subunits, i.e. two chromatids and their two chromatic halves. Eight fibrils are observed in each chromatid (16 in the total chromosome) in the leptotene chromosome of Tradescantia. Each fibril is about 200 Å in diameter. These fibres are seen as twisted around each other two by two. These, however, are not the finest unit—the meiotic prophase chromosomes in many plants are found to contain microfibrils even 100 to 120 Å in diameter. In certain cases, there is further subdivision of each of these two fibrils into fibrils having a mean diameter of 20 to 40 Å. So far, this is the lowest unit recorded and it may represent the absolute lowest limit. There is a possibility that each of these represents one double-helix DNA molecule. These are termed as subfibrils.

## **Chemical Composition of Chromosomes**

DNA, RNA, proteins and lipids are the four principal chemical compounds in the nucleus. In the chromosome, DNA and histone, a basic protein, are bonded to form a deoxiribonucleoprotein which is about 90% in most of the organisms. When DNA and histone are extracted from the chromosome using the enzymes DNase and protease respectively, the substrate of the chromosome is observed as a fine thread made up mostly of residual protein, RNA and a small amount of DNA. This substrate is called the *residual chromosome*. The residual protein is an acidic protein containing large amounts of amino acids such as tryptophan and tyrosine. Proteins serve as the skeletal structures of chromosomes to which the nucleic acid is attached. The fact that when the residual proteins are removed, the structure of chromosomes disintegrates, confirms this.

DNA and protein form salt-like compounds. The nature of the bond is ionic since the positively-charged radical histone, for example, combines with the negatively-charged radical of nucleic acid. Besides these salt linkages, it has been experimentally shown that bivalent metallic ions such as Ca, Fe and Mg are involved for bonds between DNA and protein or between DNA groups themselves.

Cytophotometrical studies indicate that the amount of DNA present is directly related to the number of chromosomes. The somatic nuclei of all the cells of diploid species contain twice the amount of DNA as compared to that in the haploid nuclei of mature sperms and eggs of that species. This constancy in the DNA amount in relation to the number of chromosomes has led to the view that DNA is almost identical in all species. However, DNA from a particular species is specific and it brings out the development of specific characters. This specificity is on account of almost infinite permutations and combinations of the carboxyl and amino groups of the amino acids. The accepted model of the structure of DNA as proposed by Watson and Crick has been dealt later on in this book. Genes are also said to be constituted in a linear fashion along the entire length of each chromosome.

DNAs of plants are characterised by having a relatively high content of 5-methyl cytosine (Thomas and Sherrott, 1956). Histones isolated from plant sources have an amino acid composition similar to comparable fractions obtained from calf thymus.

In meiotic chromosomes of plants as well as animals, paired strands of dense material about 300 Å wide and 1500 Å apart are observed during zygotene and pachytene, i.e. during synapsis. Hence, they are called *synaptinemal complex*. This is a consisting feature. The synaptinemal complex is not, however, found in organisms in which although there is a pairing of chromosomes, there is no crossing over (Moses, 1964). This fact indicates that the synaptinemal complex must have some special significance in chiasma formation. It has been reported in plants during microsporogenesis as well as megasporogenesis (Israel and Sagawa, 1964; Menzel and Price, 1966; Lu, 1966). It is suggested that the dense strands represent the axes of the meiotic chromosomes and that the thin line which is seen sometimes midway between the paired strands is the junction of the two chromosomes that form the bivalent.

We know therefore that chromosomes are composed of DNA, RNA, histones and acidic proteins but the manner in which the nucleoprotein complex is assembled is not clear. It is assumed that RNA is localised at certain regions of the chromosome while proteins are associated with the DNA molecule along its entire length. The term 'gene' is hypothetical. It has no physical entity. It is not visible even under an electron microscope. We shall discuss the gene concept later on.

Lipids (phospholipids) bonded with proteins also occur in small quantities in the chromatin.

#### **Chromosome Models**

In order to explain the association of proteins with DNA, several models have been proposed. Before we deal with some of these models, it is first necessary to know the controversial aspects in these models. There are three main aspects: (i) Whether the chromosome contains a single strand or multiple strands of DNA. (ii) Whether DNA is present as a continuous strand from one end of the chromosome to the other or interrupted by 'linkers'. (iii) In what manner are the protein associated with the DNA molecule.

#### Wilkin's Model

Wilkins suggested in 1957 that the protein chains in nucleohistone might be coiled round the DNA situated in the grooves of the double helix so as to form a nucleohistone thread of about 30 Å diameter. Wilkins et al. (1969) carried out x-ray diffraction studies on the basis of which they came to the conclusion that the nucleohistone is itself supercoiled to form a helix about 100 to 130 Å in diameter.

#### Ris' Model (Figs. 12.7 and 12.8)

According to Ris (1967), histone is associated with DNA in some regular but unspecific fashion. Some type of coiling or folding occurs again to form a nuclear histone fibre of about 100 Å. Further folding takes place because of Ca bridges, and a basic fibril of about 200 to 250 Å is formed. Ris has produced electron micrographs of both 100 Å and 250 Å fibrils. He has suggested regions of still further folding.

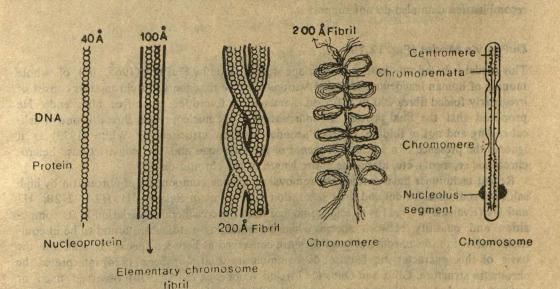


Fig. 12.7 Ris' Model.

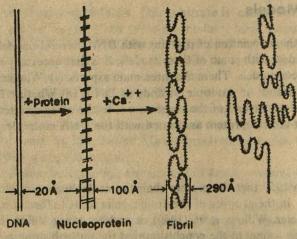


Fig. 12.8 Modified model by Ris.

## Taylor's Model (Fig. 12.9)

In 1957, Taylor proposed a model according to which the chromosome is composed of a long protein backbone from which DNA coils branch off similar to the legs of a centipede. Replication is explained on the basis of an assumption that the protein backbone is double-layered and that these layers can be pulled apart. It is thought that each layer has one strand of DNA helix on separation. On such a separated chromatid, a new chromatid could then be formed. The greatest drawback of this model is that it ignores the fact that genes are arranged in a linear fashion along the entire length of the chromosome. The genetic recombination data also do not support it.

### DuPraw's Model (Fig. 12.10)

This model is based on electron microscope studies made by DuPraw (1965, 66) of whole mounts of human leucocytes. His observations indicate that the sister chromatids consist of irregularly folded fibres 200 to 500 Å in diameter. He found few or no free fibre ends. He proposed that the final process of condensation of the nucleoprotein thread is one of helical coiling and not of folding. DuPraw's model has been criticised by White (1973) as it ignores the presence of a constant sequence of chromosomes and the constrictions, heterochromatic segments, etc. along the entire length of the chromosome.

Recent techniques have enabled the removal of histone components of chromatin by high salt (2M sodium chloride) and their separation into five main classes—H1, H2A, H2B, H3 and H4 (Evans, 1981). All is rich in lysine and there is considerable variation in its composition and quantity between species while the remaining histones are found to be in equimolar ratios in most chromatins and they are conserved as between species. It was on the basis of this characteristic feature of equimolarity that Kornberg (1974) interpreted the chromatin structure. Olins and Olins (1974) and Woodcock et al. (1976) observed fixed or unfixed spread chromatin appearing under the electron microscope as a mesh of single 20 Å fibres connecting up blobs of protein.

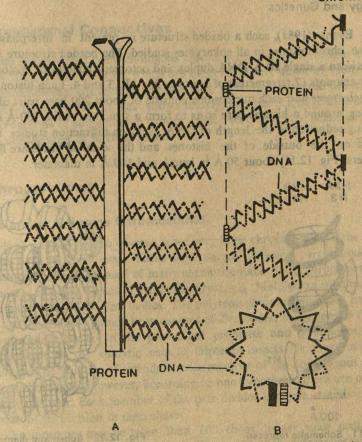


Fig. 12.9 A-Taylor's centripede model. B-Freese-Taylor's model.

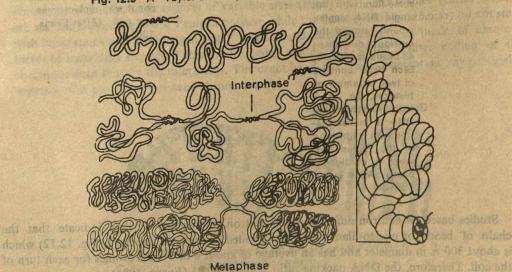


Fig. 12.10 DuPraw's folded fibre model.

According to Evans (1981), such a beaded structure is present in interphase chromatin and metaphase chromosomes in all eukaryotes studied. The beaded structure represents an association between a single 20-Å DNA duplex and octomeric spheres of histones containing equimolar amounts (2 tetrameres) of histones 2A, 2B, 3 and 4. Each histone DNA bead is termed a nucleosome and each nucleosome consists of about 200 base pairs of DNA coiled about 2½ times around a histone core so as to form a structure with a 100-Å diameter and a 7-fold packing reduction in the length of DNA. Neutron diffraction studies indicate that DNA is coiled on the outside of the histones and the so-called beads are flattened disc shaped cylinders (Fig. 12.11) about 50 Å in length and 100 Å in diameter.

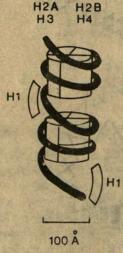


Fig. 12.11 Schematic representation of the nucleosome fibre with the single DNA duplex wound around the outside of disc-shaped beads of histone. Each bead consists of two tetramers of histories, 2A, 2B, 3 and 4. The fibre DNA is in association with H1 histone in the region betcontiguous ween beads. (After Evans, 1981.)

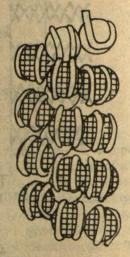


Fig. 12.12 Schematic diagram of a solenoid structure with about six nucleosomes per coil. (After Evans, 1981.)

Studies based on neutron diffraction and electron microscopy further indicate that the chain of beads or nucleo-filament is itself a coiled or solenoid structure (Fig. 12.12) which is about 300 Å in diameter and has an average of about 6 or 7 nucleosomes for each turn of the coil. Therefore, the DNA packing ratio is about 40:1 (Evans, 1981).

## Interchromosomal Connectives

Recent studies indicate that nonhomologous chromosomes within a nucleus are interconnected by very thin fibres of DNA which are observable only under an electron microscope. These interchromosomal connections are assumed to be the structural branches of chromosomal DNA. Lampert (1971) has shown clearly the presence of these connections in the electron-micrographs of some human cancer cells. According to Schneider (1972), these connections are the sites of RNA synthesis. transfer at the least to the first production of the feature of the last to the action of

#### **Giant Chromosomes**

## Salivary Gland or Polytene Chromosomes

Balbiani (1881) was the first to observe some very large chromosomes in the cells of salivary glands of the larva of Chironemus. These were named polytene chromosomes by Kollikar since they seemed to be composed of many chromonemata. Such chromosomes were subsequently reported in other tissues of Dipteran larvae, e.g. Malpighian tubules, epithelial lining of the gut, and some fat bodies. The polytene chromosomes that have been studied intensively are the salivary gland chromosomes of Drosophila melanogaster (Fig. 12.13).

Polytene chromosomes are in the permanent prophase and homologous chromosomes are in pairs even in the somatic cells of Dipteran insects. This type of pairing is called somatic pairing. The number of strands in each member of the homologous pair varies from 500 to several thousands. However, according to one hypothesis, there are only four strands in the homologous pair. Each member of the pair divides into two strands in the prophase in anticipation of its division in metaphase.

Polytene chromosomes may be more than 100 times longer than ordinary metaphase somatic chromosomes. Their characteristic feature is their differentiation into a series of chromatic and achromatic regions of variable sizes (Fig. 12.14). The former (called bands), are stained intensely and are Feulgen positive while the latter (called interbands) do not stain with basic stains and are Feulgen negative. The bands are assumed to be made of chromomeres of individual strands. These are formed probably due to the tighter coiling of the chromonemata than in the interbands. The exact band pattern is constant in any particular segment of a particular polytene chromosome.



Fig. 12.14 Part of the fourth polytene chromosome of Drosophila melanogaster.

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Bands are rich in DNA. They also contain a certain amount of RNA and basic proteins. Interbands are poor in DNA and contain acid proteins instead of basic proteins. However, they possess greater elasticity than bands.

There is evidence that the banding profiles of chromosomes may represent in part differences in the base composition along the entire length of the chromosome. Recent studies indicate that the protein components of chromosomes are involved in producing the banded structure (Evans, 1981).

It is assumed on the basis of genetic experiments that some genes are located in specific bands, although there is no direct morphological evidence of this. Cytological and genetic maps of *Drosophila melanogaster* and several other species, however, have shown the presence of specific gene loci in the bands.

#### Chromosome Puffs

Although there has been no intensive study of interbands, their 'puffing out' during larval development indicates their correlation with certain gene loci.

The phenomenon of swelling or puffing of some bands and interbands has been noted during the development of larva in several species of Diptera. Some bands and interbands are defined sharply while others exhibit diffuse swelling or puffs. It appears that the chromonemata in the puff regions are spun out laterally and hence, the increase in the diameter of the chromosome in these regions. Several regions in the polytene chromosomes may be involved in this puff formation. Some puffs are markedly larger than others. These are called *Balbiani rings* (Fig. 12.15), after Balbiani who discovered them in *Chironemus* in 1881. These rings appear fluffy or blunted under the electron microscope.

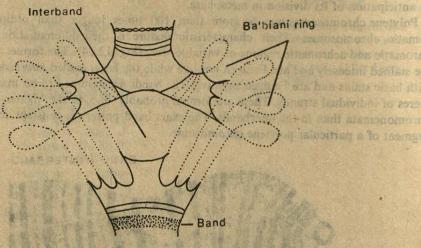


Fig. 12.15 Balbiani ring in the polytene chromosome.

It is suggested that the appearance of a puff is an expression of a specific genetic activity at a specific time. This is supported by the following facts: (i) A large amount of RNA is present in the puffs. The bands without puffs contain mainly DNA and histone. (ii) Injection

of uridine into Chironemus larvae and subsequent autoradiographs indicate RNA formation in puffs while very little activity is seen in the remaining chromosome and cytoplasm. (iii) When radioactive leucine is injected, no radioactive material is taken by proteins indicating that puff formation does not take place in the chromosome but elsewhere. (iv) RNA in the puff is the messenger RNA (mRNA) and puffs are active in its synthesis but not in protein synthesis. mRNA is synthesised in the puffs using a DNA template.

It has been observed that the larvae of Diptera constantly feed themselves and their salivary glands are very active in secretion. Hence, it seems probable that metabolic changes

involved in puffing have a close relation to secretory functions.

# Lampbrush Chromosomes (Fig. 12.16)

It has been observed that the nuclei of many vertebrate oocytes, especially those of amphibia, possess chromosomes which are longer (more than  $1000\,\mu$ ) at their maximum development as compared to the longest polytene chromosome. In some salamanders, they may attain a length of  $5900\,\mu$ . They are about  $20\,\mu$  broad during early prophase-I. The number of chromonemata, however, in these chromosomes is just the same as in the typical chromosomes. In late prophase, the first meiotic division, they attain their maximum development. At this stage, they are characterised by paired loops originating from the main axis of the chromosome, which are very much like the hairs of a lampbrush. These chromosomes have also been noted in plants (Grun, 1948). In the next metaphase-I, lampbrush chromosomes lose their lampbrush appearance and come back to their normal form.

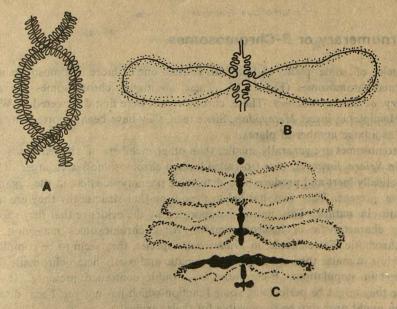


Fig. 12.16 Diagrams of lampbrush chromosome of oocyte of *Triturus*.

A—Under low magnification. B—Under high magnification.
Chromonema coiling and two lateral loops can be seen.
C—Same, showing distinct difference between loops and chromomere.

The central axis of a lampbrush chromosome probably consists of at least four chromatids to which the lateral loops are attached. The loops are the laterally extended portions of the chromatids. As they are covered with matrix they look fuzzy. Ris (1959) has observed that the loops are bundles of submicroscopic fibrils. The chromosomes are situated at the base of the loops. They take a dark stain and represent tightly coiled regions of the chromosome axis. The diameter of the loop axis is estimated to be 30 to 50 Å. It was Gall (1958) who first mentioned the occurrence of a certain number of DNA strands in each lampbrush chromosome. It is now established that the axial filaments and chromosomeres are composed of DNA. There are small swellings without loops at the end of the axial filaments. These swellings are telomeres. There is also a swelling contained in each bivalent. This is free of loops and is called the centromere. Miller and Beatty (1969) made an electron micrograph study of lampbrush chromosomes of the oocytes of the salamander Triturus viridescence and found the presence of dense granules on the DNA axial fibre. These granules are considered to be large molecules of the enzyme RNA polymerase involved in RNA synthesis. From these enzyme molecules, there arise fine fibrils of ribonucleoproteins. The loop is regarded as representing one long operon consisting of a series of identical copies of the same structural genes. According to Callan and Lloyd (1960), each pair of loops is associated with the activity of a specific gene. Each loop is believed to contain repeating sequences, i.e. genes in a series. Therefore it appears that loops are concerned with RNA and protein synthesis. It is believed that the lampbrush chromosomes are also involved in the formation of a certain amount of yolk material for the egg.

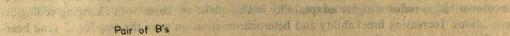
# Supernumerary or B-Chromosomes

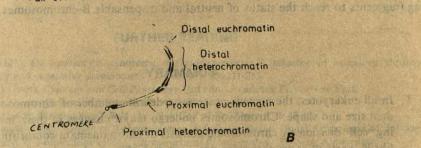
The nuclei of some plants and animals contain one or more chromosomes in addition to the normal chromosomes (Fig. 12.17). These additional chromosomes are called *supernumerary or B-chromosomes*. These chromosomes were first discovered by Wilson in 1905 in the Hemipteran insect *Metapodius*. Since then, they have been reported in many insects as well as a large number of plants.

B-chromosomes are generally smaller than other members of the chromosomal complement. In Sciara, however, they (so-called limited chromosomes) are larger. They seem to be genetically inert and produce little detectable phenotypic effect in the organism in which they are present. This fact has led to the notion that structurally they are mostly heterochromatic in nature. This notion is supported by the evidence that they show differential staining characteristic of heterochromatin. All B-chromosomes may not, however, be heterochromatic. For example, in Tradescantia species, they seem to be completely euchromatic while in maize they are partly euchromatic and partly heterochromatic (Fig. 12.18).

In certain populations, B-chromosomes exhibit continued presence. It is, therefore, possible they might be performing some function which has not yet been discovered. This function might probably be responsible for their survival.

Bosemark (1957) has done considerable work on B-chromosomes in grasses such as Alopecurus pratensis, Briza media, Festuca arundinacea, Holeus lanatus, Phleum nodosum and Poa trivialis. He has observed that B-chromosomes in these plants are smaller than normal ones and easily distinguishable. They are very likely to be entirely heterochromatic.





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Fig. 12.18 Morphology of B—chromosome in maize, A—Photomicrograph, B—Camera lucida drawing (Courtesy: Dr. S.H. Tulpule.)

The results of crosses indicate that the mechanism of numerical increase is restricted to the male side in all the species. His study suggests that in the case of certain genotypes, under certain environmental conditions, accessory chromosomes may be useful.

In maize (Randolph, 1941a), 25 to 30 B-chromosomes may be present in a single plant. Randolph has observed that when too many B-chromosomes are present, there is some reduction in vigour and fertility. Pingle and Dnyansager (1976) also noted a deleterious effect in respect of pollen fertility and seed output in plants of Solanum viarum (syn. S. khasianum var. chatterjeeanum) possessing one or two B-chromosomes. Therefore, it seems that they are not genetically inert.

B-chromosomes as a group are relatively unstable members of a chromosome complement. They exhibit certain peculiarities of segregation in the micropores of plants. In meiosis also their segregation is irregular. They undergo somatic nondisjunction and elimination and often show morphological changes through fragmentation.

Although the ancestry of B-chromosomes is almost unknown, all workers dealing with them agree to the point that the origin of accessory chromosomes is from the normal complement of chromosomes. In most of the animals and some of the plants, the origin of

B-chromosomes from the heterochromatic Y-chromosomes has been suggested because of their ability to disturb the sexual balance wherever they are present. In most plants where differentiation of autosomes and sex chromosomes is not evident, heterochromatisation of B-chromosomes and the loss of pairing with the normal complement at meiosis during the course of evolution elude all hopes to assess their origin (Dnyansagar and Pingle, 1979).

The nature or origin of B-chromosomes has been traced with certainty to some extent in some organisms. For example, in *Metapodium*, it seems that they are derived from the Y-chromosome. Dhanaraj(1971) reported one or two B-chromosomes in *Solanum khasianum* var. chatterjeeanum (Fig. 12.17) for the first time (normal 2n = 24). Dnyansagar and Pingle (1979) found the percentage of plants with one or two chromosomes to be 13.29 and 7.43 respectively. They observed that the presence of a fragment in the pollen grain changed the behaviour of self-incompatibility to self-compatibility in *Solanum viarum*. According to them, the change in the behaviour along with variability in the flowering period due to fragment bearing S-genes (genes concerned with self-compatibility and self-incompatibility) induces higher adaptability in this plant to cope with changing ecological conditions. Increasing breakability and heterochromatisation pave the way for S-gene bearing fragments to reach the status of neutral and dispensable B-chromosomes.

#### SUMMARY

- In all eukaryotes, the nucleus possesses a definite number of chromosomes of individual size and shape. Chromosomes undergo major changes in their morphology during cell division. A chromosome consists of chromonemata comprising many microfibrils. Parts of some chromosomes, seen as highly condensed areas in the interphase nucleus, are called chromocentres (prochromosomes). These represent tightly coiled chromosome regions.
- 2. The term 'chromatin' is used for the DNA-containing structure of the nucleus. The regions of chromatin which are stained darker or weaker than others are called heterochromatic regions. Such a difference in staining is called heteropyknosis and the regions which do not exhibit it are termed euchromatic regions. Small beadlike bodies observed in the heterochromatic regions are known as chromomeres. DNA is more labile in heterochromatin.
- 3. Euchromatin is that part of the chromosome which can be demonstrated to contain genes by classical methods of genetic analysis.
- 4. The size of chromosomes is generally constant. Depending upon the position of the kinetochore (primary constriction), chromosomes are classified into four types: acrocentric, submeracentric, metacentric and telocentric. There are chromosomes in which the secondary constriction marks the formation of a round or elongated body called the satellite. Each chromosome contains at least four sub-units, i.e. two chromatids each with two chromatid halves. Every linear sub-unit can be referred to as a chromonema. Two or more chromosomal fibres are coiled together in a paranemical or plectonemical fashion. A meiotic chromosome consists of a major coil and a minor coil.

- 5. In chromosomes, DNA and histone are bonded to form a deoxyribonucleoprotein. The quantity of DNA is directly related to the number of chromosomes. In order to explain the association of protein with DNA, several models have been proposed.
- 6. Salivary gland or polytene chromosomes are very large chromosomes observed in Dipteran larvae. They are multistranded structures. Their characteristic feature is their differentiation into a series of chromatic (bands) and achromatic (interbands) regions. The phenomenon of swelling or puffing of some bands and interbands is seen during the development of larva of several species of Diptera. The nuclei of many vertebrate oocytes possess chromosomes which are longer compared to the longest polytene chromosome.
- 7. In the late prophase of meiosis-I, they are characterised by paired loops originating from the main axis of the chromosome and look very much like the hairs of lamp-brush and so are called lampbrush chromosomes. In some plants or animals, nuclei contain one or more chromosomes in addition to the normal chromosomes. These are termed supernumerary or B-chromosomes. They are mostly heterochromatic in nature.

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# 13. Cell Division

The growth and development of an organism depends on the growth and multiplication of its cells. A unicellular organism reproduces by cell division. In this process, one or more individuals are formed from the mother cell. However, in the case of multicellular organisms, the zygote develops into an adult by the division of cells. It is extremely difficult to define growth exactly. The other aspect of growth is differentiation, which takes place either before or after cell division and increase in cell size. The cytoplasm becomes functionally differentiated before cell division. A cell increases in size after division and in the case of plant cells, there is then differentiation in the structure of their cell walls and chemical organisation. Several scientists limit the term 'growth' to the processes of cell division and growth and use the term 'differentiation' for the changes that take place in structure and chemical composition.

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Flemming (1879) classified nuclear division into two kinds: direct (amitosis) and indirect (mitosis). In the former, the nucleus remains intact. It only constricts into two pieces so as to assume a dumb-bell shaped form and then cytoplasm follows its division. The process of indirect division is, however, complicated.

Although mitosis means just nuclear division and does not include the division of cytoplasm [for which Whitman (1887) proposed the term cytokinesis], in practice, mitosis includes nuclear as well as cytoplasmic divisions. When it occurs, there is sorting out of cytoplasmic organelles such as mitochondria and Golgi complex into approximately two equal parts to the daughter cells. Such a distribution of mitochondria is termed chondriokinesis and of the Golgi complex dicytokinesis.

There are two common forms of mitosis: somatic and meiotic. In somatic mitosis, new cells are formed in the growing regions of the organisms but the chromosome number remains constant. In meiotic division, the chromosome number is reduced to half and it results directly or indirectly in the formation of sexual gametes. There is a third form of mitotic activity seen usually in differentiated tissues. It is called *endomitosis*. In this type of division, there is progressive duplication of chromosomes without any cell division. The usual mitotic stages are absent.

Although cell division may vary from one species to another, the process and consequences are fundamentally similar in all organisms. Cell division enables an organism to increase the number of cells. It gives rise to gametes which take part in sexual reproduction—a means to increase its number. Since chromosomes, the bearers of hereditary characters, take part in cell division, there is both qualitative and quantitative distribution of the genes located in chromosomes among the daughter cells.

For the sake of convenience, cytologists divide the process of cell division into five

stages: interphase, prophase, prometaphase, metaphase, anaphase and telophase. But it should be borne in mind that cell division is a dynamic and continuous process and each stage passes almost imperceptably into the next, with the exception of metaphase and anaphase, which can be defined easily since they appear as markedly discontinuous stages.

#### Mitosis

The most convenient material for the study of mitosis (Figs. 13.1 to 13.3) are the root tips of germinating seeds such as onion or maize. The material is first fixed in Cornoy's fluid and subsequently embedded in paraffin wax for preparing microtome sections either transversely or longitudinally. It is then stained with a basic dye (e.g. Feulgen or haematoxylin). The common practice is to make a squash preparation by pressing a small piece of fixed root tip on a slide and then staining with Feulgen (specific for DNA in chromosomes) or acetocarmine. The temporary preparation after examination of division stages is made permanent.

Cell division is a cyclical process and one can begin at any point. We shall, however, first

deal with interphase (Figs. 13.1 to 13.3).

#### Interphase

In actual fact, the process of mitosis does not take place during interphase. It is just a time interval between the end of telophase and commencement of the next prophase. There is variation in its duration from organism to organism.

Interphase represents a critical period during which the cell prepares itself for division. The important events that are connected with mitosis are the division of the centriole (in animal and some lower plants), replication of chromosomes, and the synthesis of proteins required for spindle formation. Interphase is divided into three substages: G1, S and G2, on the basis of synthetic activities. During the G1 substage, there occurs synthesis and organisation of substrate (RNA) and formation of enzymes (proteins) required for DNA synthesis. It is most variable in duration and takes about 25 to 50% of the interphase. During the S-phase, DNA synthesis takes place and the DNA content of the nucleus gets doubled. This phase is relatively of constant duration in case of similar cells of a species. Its duration is about 35 to 40% of the interphase time. The S substage is followed by the G2 substage during which there is occurrence of some organisational events which lead to mitosis. In early G2 substage, ribosomes required for subsequent cell cycle, and the proteins necessary for spindle formation are also produced.

During the G1 substage, chromosomes are generally observed as single-stranded structures, while in G2 substage they become double-stranded, possessing two chromatids each. This is because of DNA duplication during the S substage. In other words, the nucleus which has 2c value before DNA synthesis assumes 4c value after its synthesis. Histone proteins are also synthesised along with DNA. The synthesis of some of them, however, is completely stopped during late prophase and it takes place again at some time during states of office a lateral flat to botton are unoded of the or way in

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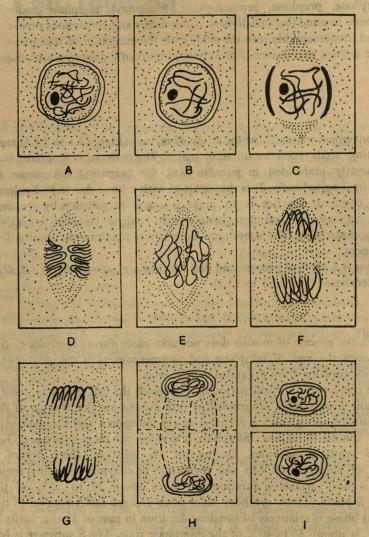


Fig. 13.1 Stages in mitosis (diagrammatic) in a plant cell.

A—Early prophases. B—Late prophase. C—Early
metaphase. D—Metaphase. E—Early anaphase. F—
Late anaphase. G—Telophase. H—Telophase, formation of daughter nuclei and cell plate. I—Cytokinesis.

#### Early Prophase 150% suite 1500 and 1500

A lot of significant changes occur in the nucleus as well as cytoplasm during prophase. There is condensation of chromosomes and this process continues throughout the successive stages of mitosis. It appears that two factors are involved in the consideration of chromosomes. Chromosomes expand during interphase and are in a highly hydrated gel form.

There is at first loss of water from the gel and then progressive spiralisation of the thread takes place. There is association of many molecules of water with each molecule of nucleoprotein. It is possible that there is deposition of nucleoprotein. It is possible that there is deposition of RNA on the chromosome strand when there is condensation of the latter. The spiralisation of chromosomes has been shown clearly in plants by several workers. It takes place in two stages; primary and secondary coiling. In the former, a helical structure is formed by the long strands of the chromosome. Once this is achieved, large secondary coils can be formed by the spiralised chromosomes. At this stage, chromosomes are doubled and it is necessary that coiling does not come in the way of the daughter chromosomes at metaphase.

We have already seen that chromosomes appear double-stranded in early prophase as their replication had taken place during interphase. The two strands spiralise independently and remain adjacent to each other. The separate strands of the chromosome are termed chromatids.

When the process of condensation of chromosomes starts, the distinction between heterochromatin and euchromatin of the chromosome begins to become less and as the process continues, it is finally lost. It is possible that part of RNA which is so released gets attached to other parts of the chromosomes. The nucleolus which was serving as the functional unit of the cell during interphase is not seen as a highly organised structure.

While the above mentioned changes are occurring in the nucleus, the cytoplasm also undergoes certain changes. In animal and some lower plant cells, there are centrioles situated within the cytoplasm adjacent to the nuclear membrane, whose structure we have already studied in Chapter 10. Each dividing cell possesses two centrioles. In the case of ciliated epithelial cells, there is replication of centrioles resulting in the formation of basal bodies from which cilia arise. The spindle is first formed around the centrioles. It develops from the rounded bodies situated around the centriole. When the spindle fibres start to form, they radiate in an outward direction from the centrioles so as to give a starlike appearance. Hence the assembly of radiating fibres of the spindle is called an aster. In plant cells, the spindle is less distinct in the early stages of its formation as compared to animal cells. In the case of the former cells, it does not get organised until the nuclear membrane breaks.

#### **Late Prophase**

In animal cells, the spindle develops and the asters move apart in late prophase. There is also disintegration of the nuclear membrane. It seems that in the endosperm cells, the nuclear membrane breaks up into small liquid droplets. As a consequence, there is mixing of the nuclear sap and cytoplasm. What we see as fibres now under the light microscope, were regarded for many years as a part of the spindle. However, polarizing and electron microscope studies indicate that these so-called fibres are, in fact, an artefact of fixation produced by precipitation. There is, however, strong proof that the oriented structures do exist in the mitotic spindle in living cells.

The researches carried out with the aid of an electron microscope indicate that the spindle fibres are formed by the aggregation of much smaller fibres called microtubules. These are straight and lie in parallel bundles. Their outer diameter is 200 to 270 Å and the thickness of the wall is 50 to 70 Å. They are made up of 13 longitudinal filaments spaced 55 to 60 Å apart. The radiating microtubules arise from the centriole. They are associated with the microtubules in the late prophase. The spindle in living cells can be observed clearly under a sensitive polarizing microscope.

# Prometaphase

Prometaphase commences with the disintegration of the nuclear membrane. There appears a more fluid zone in the centre of the cell. Chromosomes move freely in this zone in apparent disorder towards the equator.

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Metaphase begins when the chromosomes reach the plane of the equator and arrange themselves radially at the periphery of the spindle. The arrangement of chromosomes at metaphase in plant cells is irregular. They occupy the entire surface of the equatorial plane of the spindle. If both the small and large chromosomes are present in the group, the former are usually observed towards the interior while the latter are situated at the periphery.

The display of chromosomes on the spindle is called the equatorial plate. The kineto-chores connect the chromosomes to the spindle fibres. The fibres which connect to the chromosomes are called chromosomal fibres and those which extend from one pole to the other without hindrance are called continuous fibres. For determining the number of chromosomes and studying their morphology, metaphase chromosomes in polar view are the best material.

At metaphase, there is equilibrium of forces. This equilibrium is broken by the division of the kinetochores which had held together two chromatids of the chromosome. This division takes place simultaneously in all the chromosomes and the resulting daughter kinetochores move apart. In consequence, chromatids get separated and their movement towards respective poles starts. This process gives a hint of the commencement of anaphase.

#### Anaphase

The chromatids are now referred to as daughter chromosomes. They become shorter and separate. During the latter half of anaphase, the spindle fibres in the zone between two groups of chromosomes become attached. These fibres are known as *interzonal fibres*. The daughter chromosomes of each group start migrating to their respective poles.

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Telophase begins when two daughter groups reach the respective poles. At this stage, good micropreparations show the spiralised structure of chromosomes with their chromonemata. Thin chromatic filaments coiled around the chromosome can now be clearly seen. After a short while, the process of reconstitution of nucleus starts. This can be regarded as a prophase process in reverse. The chromosomes become less compact and the chromonemata

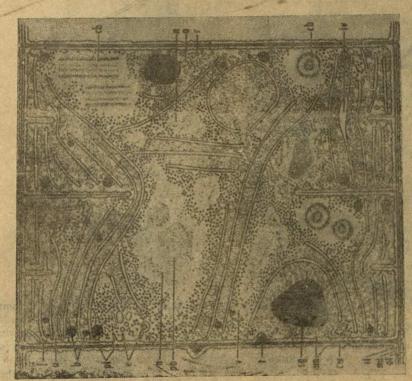


Fig. 2.4 Diagram of a median section of a cell of Symploca muscorum.

a—a granules; b—β granules; cb—cylindrical bodies; cw—cross wall; e—elaboration of plasma membrane; il, ml, ol—inner, middle and outer layer respectively, of inner investment; iv—interlamellar vesicle; I—lamellae; n—nucleoplasm; p—pores; pb—polyhedral bodies; pl—plasmodesmata; pm—plasma membrane; r—ribosomes; s—sheath; sg—structural granules; t—local thickening; v—vacuole-like inclusions (after Pancratz and Brown, Amer. J. Bot., Vol. 50, 1963, with permission of American Journal of Botany).

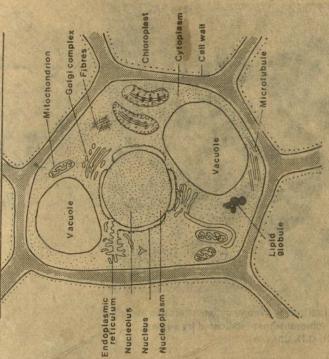


Fig. 2.5 Generalised diagram of a plant cell (redrawn after Loewy and Siekevitz, Cell Structure and Function, 1969).



Fig. 12.4 Metaphase of mitosis in *Chlorophytum tuberosum* showing a pair of SAT-chromosomes (indicated by arrows).

(*Courtesy*: Dr. C.D. Chowta).



Fig. 12.6 Coiling in meiotic (diplotene) chromosomes of grasshopper testes. (Courtesy: Dr. A.K. Indurkar).

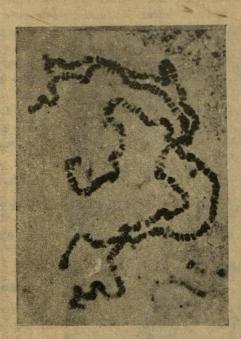


Fig. 12.13 Photomicrograph of salivary gland chromosomes of *Drosophila*. (*Courtesy*: Dr. A.K. Indurkar).



Fig. 12.17 A—Photomicrograph of somatic chromosome number 2n=24 in normal plant of Solanum khasianum var. chatterjeeanum (syn. S. viarum). B—Same, with 2n=25. B—chromosome is shown by an arrow. (Courtesy: Dr. A. Dhanaraj and Dr. A.R. Pingle).

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PLATE 3

Fig. 13.2 Photomicrographs of stages in mitosis in root tip of barley. A—Interphase, B—Prophase, C—Metaphase, D—Anaphase. E—Telophase, F—Formation of two nuclei. G—Cytokinesis. (Courtesy; Dr. R.J. Thengane).

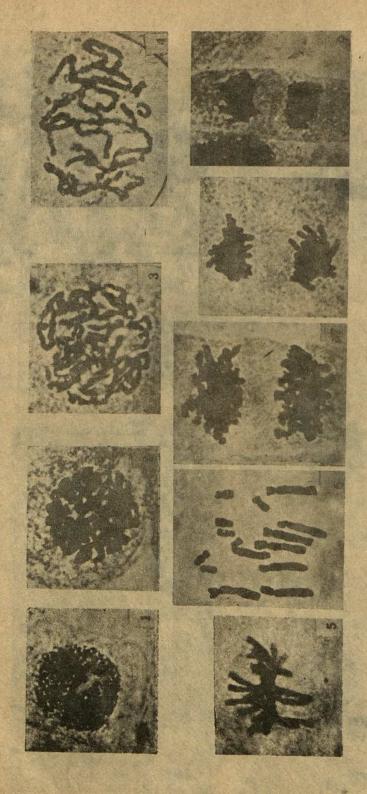


Fig. 13.3A Photomicrographs of stages in mitosis in Aloe berbadensis. 1-Interphase. 2-4-Prophase. 5, 6-Metaphase. 7-Anaphase. 8-Telophase. 9-Cytokinesis. (Courtesy: Dr. A.B. Sapre).

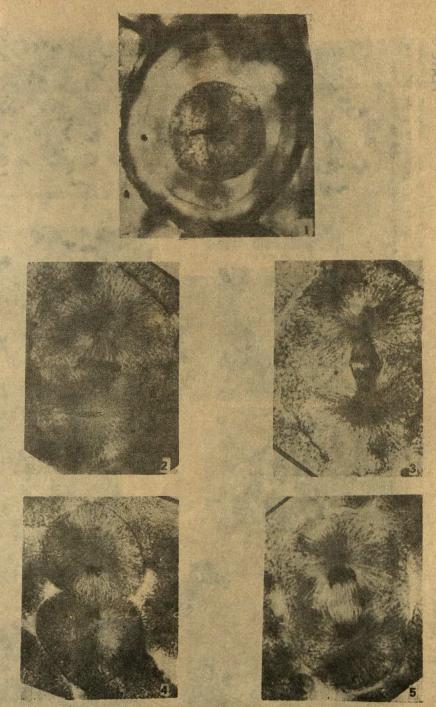


Fig. 13.3B Photomicrographs of stages in mitosis of an animal cell. 1—Cleavage mitosis in *Ascaris* showing metaphase. Note centrioles. 2-5-White fish blastula mitosis. 2-Metaphase. Spindle and asters are seen clearly. 3-Early anaphase. 4-Late anaphase. 5-Cytokinesis. (*Courtesy*: Dr. A.K. Indurkar).

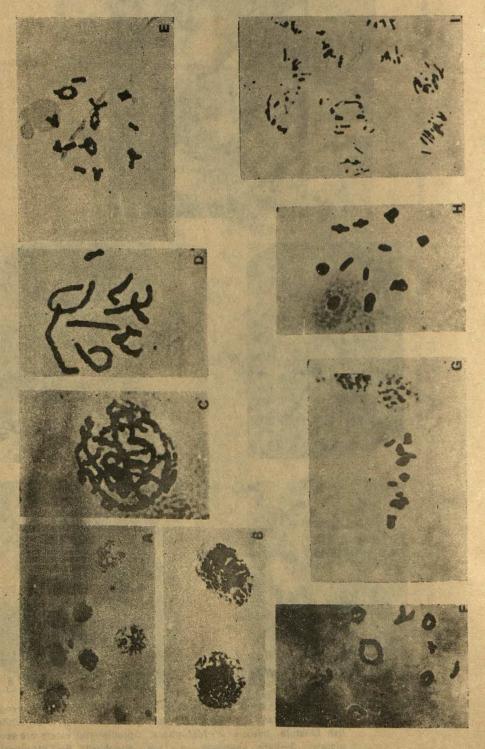
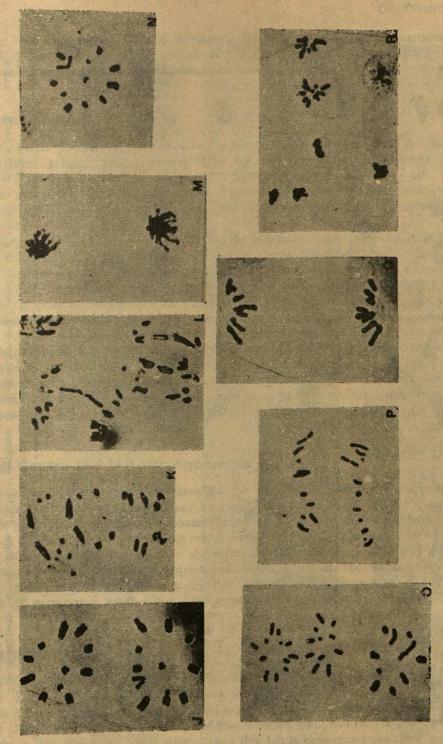
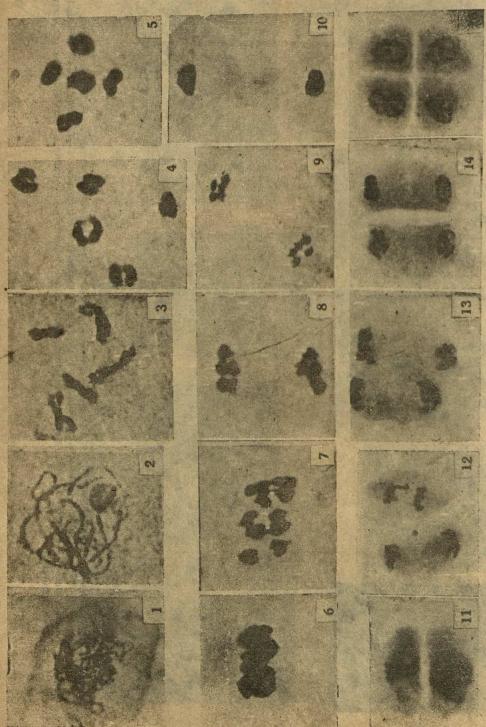


Fig. 13.5 Photomicrographs of stages in meiosis in testes of grasshopper. A-M-Meiosis-I. A-Leptotene. B-Zygotene. C-Pachytene. D-Early diplotene. E-Late diplotene. F-Diakinesis. G-Metaphase-I, equtional view. H-Same, polar view. I-Metaphase, anaphase.



group with heteropycnotic X—chromosome going to one pole, L—Anaphase, bridge formation. M—Telophase. N-R—Meiosis-II. N—Metaphase. O—Metaphase and Telophase. (Courtesy: Dr. A.K. Indurkar). Fig. 13.5 J-Anaphase V-shaped chromosome is X. Note one group is with 12 and the other with 11 chromosomes. K-Same. Note the

PLATE 8



Meiosis in PMCs of Coix aquantica. 1-10-Meiosis-I. 1-Leptotene. 2-Zygotene. 3-Diplotene and chiasma formation. 4-Diakinesis and terminalization of chiasmata. 5-6-Metaphase. 7, 8-Anaphase. 9,10-Telophase. 11-14-Meiosis. 11--Metaphase. 12-Anaphase. 13, 14-Telophase, 15-Cytokinesis and formation of a tetrad of micros pores. (Courtesy: Dr. A.B. Sapre). Fig. 13.6

coils start to unwind. There is imbibition from the surrounding nucleoplasm and the nuclear membrane is formed around each nucleus. Finally, nucleoli again appear at the site of nucleolar organisers or satellites.

# Cytokinesis

While the daughter nuclei begin to be reconstructed, cytokinesis takes place simultaneously. The latter process involves separation of the cytoplasm. In animal cells, the cytoplasm starts constricting from the equatorial region, to result in the division of the cell. In plant cells, the phragmoplast and cell plate start to form for dividing the cell and in most of the cases a cell is constituted in the region of the equatorial plate so that two daughter cells are

mo net (Figs. 13.4 17.5 os in 1905. Meyests our be divided in the following

formed (Fig. 13.1H and I).

Porter and Machado (1960) observed that the formation of the cell plate is initiated by the migration of tubular elements of the endoplasmic reticulum towards the interzonal region of the spindle. In this region, the tubular elements spread out to form a close network along the equator. This network marks the outline of the developing phragmoplast. The cell plate is formed within the latter. It is first seen as a series of unconnected vesicles within the meshes of the network. These vesicles then increase in size and fuse so that the separation of the two daughter cells is completed, except at points, where the continuity is kept by the plasmodesmata. Cell plate formation begins during late anaphase. Vesicles containing polysaccharide material are secreted from the Golgi complex which seems to act as a general mechanism for the secretion of the matrix of the cell wall. The vesicles contribute to the formation of phragmoplast which subsequently develops into the plasma membranes that are responsible for the separation of two daughter cells with a cellulose wall lying in between.

# **Cell Centre and Mitotic Apparatus**

The cell centre is a cytoplasmic organoid. It is represented either by a single or double granule, i.e. centrioles. It has already been stated that the centrioles are seen in animal and some lower plant cells in the interphase. During the mitotic process, the cell centre becomes a part of a large, highly finished structure known as the *mitotic apparatus*. It is an assemblage of structures which constitute the acromatic figure in mitosis. It includes the aster or astrosphere which surrounds the centriole and the mitotic spindle. The chief component of the mitotic apparatus appears to be a protein that is low in achromatic amino acids and contains 2 to 3% nucleic acid, mostly RNA. The chromosomal DNA content is negligible.

#### Meiosis

In 1883, Van Benedin, while working on horse threadworm (*Parascaris equorum*) observed that there were twice as many chromosomes visible during mitosis in the fertilised egg as there had been in the egg and sperm nuclei prior to the mitosis. This observation indicated that the contribution of each of male and female gametes was half the chromosome number to the zygote. Weismann (1887) inferred from this that in each generation there must occur

reduction division at some stage involving halving of the chromosome number, in the absence of which the body cells of each generation would otherwise contain twice the chromosome complement of those of the parents. Flemming (1887), Strasburger and others found that the nuclear divisions just prior to the formation of mature sperms and eggs in animals, as well as formation of pollen grains and embryo sacs in angiosperms, were different in that two nuclear divisions took place in rapid succession. The reduction division occurred during gamete formation. The entire process leading to the formation of gametes was termed meiosis (Figs. 13.4 to 13.6) in 1905. Meiosis can be divided in the following stages:

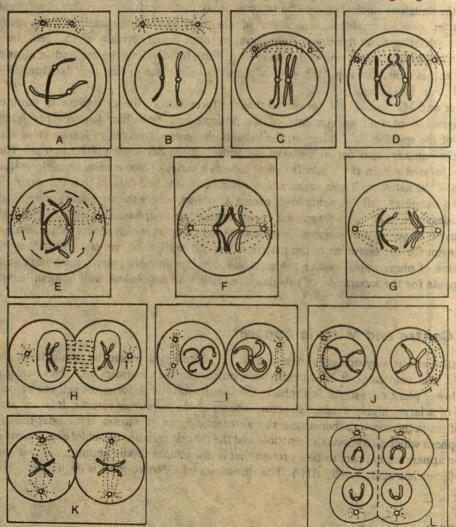


Fig. 13.4 Stages in meiosis in an animal reproductive cell (diagrammatic, only one pair of chromosomes is shown). A-J—Meiosis-I. A, B—Leptotene. C—Zygotene. D—Pachytene (chiasma formation). E—Diplotene (crossing over has occurred). F—Metaphase. G—Anaphase. H—Telophase. H and I also show that cytokinesis is taking place and cytoplasm is constricted in the region of equator. K, L—Meiosis-II.

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# Prophase-I add to do you do not be proposed to the prophase of the prophase of

Prophase-I of meiosis is generally divided into a number of stages which coincide with several phenomena that occur in association with meiosis. These stages are described below.

# Leptotene

The earliest part of leptotene is called *proleptotene*. During this phase, the chromosomes may become condensed and chromatic. But as leptotene commences, they are seen as long, uncoiled filaments. However, they cannot be identified as separate discrete threads. In early leptotene, the chromosome threads are unpaired. Later on, the chromonemata of the chromosome threads form a large number of small coils. In the chromomere regions, the chromonemata are more tightly coiled. These regions are therefore seen as highly chromatic nodes while the interchromomeric regions, i.e. the internodes, appear considerably less chromatic.

According to the majority of cytologists, the leptotene chromosomes are double. The researches of several competent workers indicate that the chromosomes are already divided in the pre-mciotic telophase in anticipation of the second meiotic division where the split that had taken place between the two chromatids becomes actually functional. It is because of this split that when the homologous chromosomes pair and synapse in the following zygotene stage, each bivalent is already divided into two. Darlington's precocious theory is, however, against this widely accepted view. According to this theory, leptotene chromosomes are not divided chromatids and hence pairing of homologous chromosomes is due to this singleness. Cytological and cytochemical evidences, however, favour the view that the replication of DNA and histone coincides with the doubling of chromosomes in the prophase, i.e. before the pairing of homologous chromosomes in the zygotene stage. The work of Ansley (1954, 57), on the contrary, supports the precocious theory since he believed that the doubling of DNA and histone takes place during zygotene in Losca flavicolis.

When prophase-I begins, the nucleolus is relatively small. Its size increases gradually during leptotene as well as zygotene. At the same time, there is a significant increase in the content of the nucleolar RNA. The nucleolus attains its maximum size by the middle of pachytene.

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## Zygotene

At the commencement of this stage, the homologous chromosomes start pairing with each other. The chromosomes may unite first at their polarized ends and then continue pairing to the antipodal extremity, or the union may occur simultaneously at various points along the length of the filament. It seems that the 'bouquet' formation and polarization aid in bringing about regularity in pairing. The pairing is exact and specific. It occurs point for point and chromomere for chromomere in each homologous chromosome. Each pair of homologous chromosomes constitutes a bivalent.

#### Pachytene

In this stage, the chromosomes contract and the members of each bivalent show a tendency to separate. The chromosomes appear double at some places. The most important biological

event which takes place during this stage is crossing over, which involves the exchange of genes between certain segments of homologous chromosomes. It appears that in certain regions, the exchange of segments between homologous half-chromosomes also occurs during this phase. An indication of this exchange is given by the X-shaped configurations (called chiasmata) seen in bivalents (Figs. 13.4D and E; 13.6C). It is generally assumed that the presence of chiasmata shows that the phenomenon of crossing over has occurred (Stern, 1931; Creighton and McClintock, 1931). There is enough evidence to show that crossing over as well as chiasma formation take place at the 4-strand stage and only two out of the four strands are involved at any locus (Figs. 13.7 and 13.8). The various theories regarding crossing over will be discussed later on.

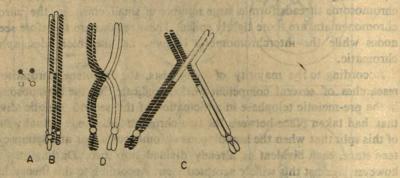


Fig. 13.7 A, B—Crossing over. C—Chiasma formation, D—Terminalization.

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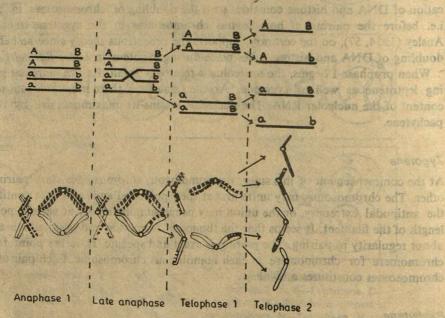


Fig. 13.8 Outline showing meiosis and result of crossing over.

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#### Diplotene

The tendency of repelling homologues in a bivalent becomes apparent during diplotene. In some organisms, the separation is complete and the result is the formation of univalents. Generally, the dissociation is not complete since the homologues are bound together by one or more chiasmata. During the process of separation, the chromosomes open out to form loops and nodes which are characteristic of the diplotene stage. The chiasmata represent the nodal regions.

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When the diplotene is coming to an end, the chiasmata start to move along the length of the chromosome from the kinetochore. This displacement of chiasmata has been termed terminalisation by Darlington (1930). Terminalisation is very well marked in a bivalent with one chiasma (Fig. 13.7D). The arms of the bivalent rotate through 180° till they assume the form of a cross. If a bivalent possesses two chiasmata, the openings formed due to the terminalisation process continue to widen resulting in the formation of a ring. If the number of chiasmata is more, the rotation of arms gives rise to the figure of a chain in which each link is perpendicular to the next. When the process of terminalisation is complete, the homologous chromosomes are held together by the terminal chiasmata. The degree of terminalisation is termed coefficient of terminalisation (T).

# $T = \frac{\text{Number of terminal chiasmata}}{\text{Total number of chiasmata}}$

The average number of chiasmata in a bivalent/bivalents of a nucleus is expressed as the frequency of chiasmata (Fq).

$$Fq = \frac{\text{Total number of chiasmata}}{\text{Total number of bivalents}}$$

Chiasmata formation does not take place in males of certain insects, e.g. Drosophila fruitfly.

#### Diakinesis

If one wants to study chromosome association, the best stage is diakinesis because in this stage the contraction of chromosomes is nearly maximum and the chromosome pairs are nicely spread throughout the cell as if there is mutual repulsion. The terminalisation is almost complete and the homologues are held together generally by the terminal chiasmata. This observation has led to the view that it is the chiasmata which hold the pairs together. However, a number of cases are known where no chiasmata are formed and yet homologues are in continued association. The nucleolus usually disappears in diakinesis. It may persist in some cases but in a reduced form till anaphase. The formation of the major coil is comany uncolling of the chronomenta during the pleted by the end of diakinesis. brom orientate at any start colling and the chira negligies that become

## Metaphase-I

Spiralisation is attained to its maximum in metaphase-I. The nuclear membrane disappears. The chromosomes move to the equator so as to become arranged on the equator. The two members of each homologous pair arrange themselves in such a manner that their kinetochores are oriented towards the opposite poles. The kinetochores of the bivalents repulse from each other and as the repulsion increases, the homologues are ready to separate. In case of

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a longer bivalent, a series of annular apertures are seen between the chiasmata in perpendicularly alternate planes. When the bivalent is short, there is only a single aperture. regard one in agent the character and the countries and the countries to the countries of the

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#### Anaphase-I

In this stage, the daughter chromatids of each homologous chromosome bound by their kinetochores begin to move towards their respective poles. There is a quick separation if the chromosomes are short since they are bound only by a terminal chiasma. If they are longer, separation is delayed because of interstitial and unterminalised chiasmata.

The composition of homologous chromosomes, i.e. paternal and maternal chromosomes, in a bivalent when separated from each other and moving in opposite directions, is different from that of the original because of the occurrence of crossing over. Two of the chromatids are of a composite nature while the other two retain their original constitution in relation to a single locus. or chiasmata is to read project of the give rice to the deare of a casin

# tion is isotropically to the metal when the process of terrology along complete Telophase-I to add catametric transport advice advanced to the semiconic regoldened

Arrival of the anaphase chromosomes at their respective poles signals the commencement of telophase-I. Chromosomes may be seen in a condensed form for some time. They may exhibit morphological characters similar to those seen in the mitotic interphase. In some cases, interphase lasts for a longer time. The average number of chican whale how

Telophase-I ends in the formation of daughter nuclei which are known as spermatocytes in males and oocyte II and the first polar body in females. In case of plants, they are called dyads, which give rise to microspore cells in the male organs and megaspores in the female organs. Charmete formeron one a care of make widown model eve Demonifactive

## Mejosis-II

This stage is very similar to mitosis, the difference being that there is no DNA duplication. The interval (interphase-II) between telophase-I and prophase-II is very short.

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# Prophase-II

This ob er and has but to are view if that is it on a made which had the ne This is similar to the prophase of mitosis except that the chromatid arms of the dyad are very much apart because of no relational coiling and the chromatids are much longer than at telophase-I, except that the chromonemata are not completely uncoiled. If there has been any uncoiling of the chromonemata during the preceding telophase-I and interphase, the chromonemata again start coiling and the chromosomes thus become shortened. The nuclear membrane disappears and the spindle is organised. If crossing over has not taken place, the dyad will have two identical chromatids, one paternal and other maternal.

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This phase lasts for a very short time. The chromosomes are arranged on the equatorial plate in such a manner that their kinetochores lie along the equator and the arms extend outwards. The kinetochores divide and the daughter chromatids become oriented towards the opposite poles.

#### Anaphase-II

In this phase, the chromatids or daughter chromosomes with their individual kinetochore start moving towards their respective poles. The chromatids appear short and thick as in anaphase-I or mitotic anaphase.

#### Telophase-II

With the daughter chromosomes having reached their respective poles, now the reconstitution of interphase nuclei begins. In plants, the cell walls are laid down (cytokinesis) so as to form four cells, i.e. a tetrad. Microspore tetrads are formed in anthers and a megaspore tetrad in an ovule. In female animals, a macrocyte known as the polar body is generally budded off after the first division and a second polar body after the second division. In other words, an egg and two or three polar bodies are formed. The end result of meiosis is the formation of four cells each containing half the somatic number of chromosomes.

#### Misdivision and Isochromosomes

Normally, the kinetochore divides longitudinally at the beginning of anaphase and moves polewards, being governed by its attachment to the spindle. It is rarely that division takes place at right angles to the longitudinal axis. However, when it does, it results in the formation of two fragment kinetochores to which each arm of the two chromatids is attached. This kind of division is called *misdivision*, and since both the arms of the chromosome are genetically identical, the chromosome is an isochromosome.

# Synaptonemal Complex

In 1956, ribbon-like 'chromosome cores' were observed by Moses, while making an electron microscope study of the Crayfish, Procambarus clarkii. In the same year, Fawcett also reported a similar structure while studying the spermatocytes of pigeon, cat and man. In 1958, Moses, while studying the spermatocytes of salamander Plethodon cinereus, found the presence of cores during synapsis. He therefore called them synaptinemal complexes (now spelt as synaptonemal). Since then cynaptonemal complexes have been reported in several animals as well as plants.

Leptotene chromosomes contain axial filaments which are later on transformed into lateral elements of the synaptonemal complex (Figs. 13.9 to 13.11). When the homologous chromosomes pair in zygotene, the complex appears and it is complete at the beginning of pachytene. In most cases, it disappears by diplotene. Some remnants of it may, however, be observed in diakinesis.

The synaptonemal complex is present between the homologues of a bivalent. It comprises a dense central element, on either side of which there is a dense lateral element. The former average of a sulgroup of mapping to

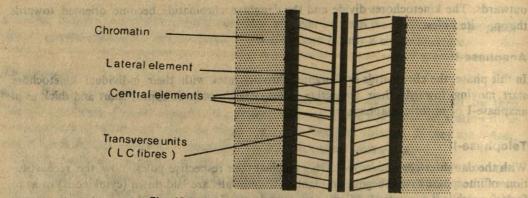


Fig. 13.9 Diagram of synaptonemal complex.

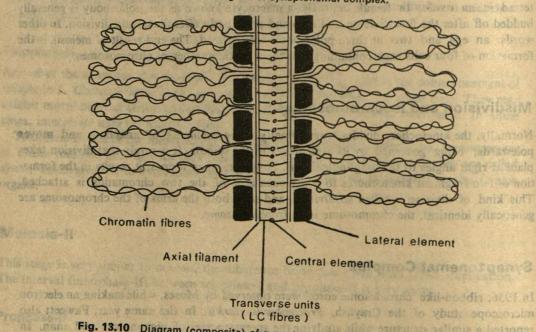


Fig. 13.10 Diagram (composite) of synaptonemal complex on the basis of work of Baker and Franchi, Moens and, Comings and Okada

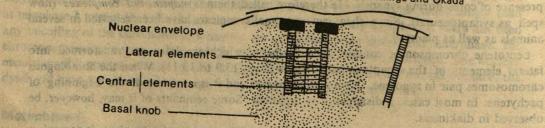


Fig. 13.11 Diagram showing synaptonemal complex to the nuclear membrane by the lateral elements.

is 150 to 500 Å and the latter 300 to 500 Å in width. The lateral elements are connected with the homologous chromosomes on its inner side. A series of transverse units (lateralcentral or LC fibres) traverse the space between the central and lateral elements. The space between two lateral elements is 900 to 1200 Å. The synaptonemal complex is connected with the inner surface of the nuclear membrane through its both ends. Comings and Okada (1971) have derived a series of lateral loops of chromatin originating from the lateral elements. A series of small loops is also given off from the lateral elements which fuse in the centre to form the central element. The transverse units are formed by the transverse portions of the loops.

Two protein filaments constitute one lateral element, one for each sister chromatid. Present evidence indicates that the central element, lateral elements and transverse units are made up of protein.

According to Meyer (1964), the synaptonemal complexes take part in chiasma formation and crossing over. However, no chiasma formation has been observed in certain organisms, though the synaptonemal complexes are present. TO THE LIGHT OF THE TEXT OF THE BOTTON OF THE SECOND OF TH

## Comparison of Mitosis and Meiosis

It is important to remember the differences in the behaviour of mitotic and meiotic chromosomes. In mitosis, the kinetochore of the metaphase chromosomes is not functionally divided. The kinetochores lie on the metaphase plate while in meiosis, the bivalents possess two undivided kinetochores from the point of function. Instead of remaining on the metaphase plate, they are arranged on the side of the long axis of the spindle. The distance between them is controlled by the proximal position of the chiasmata. At this time and before the separation of the chromosomes, repulsion is created between the homologous kinetochores. In mitosis, the kinetochores divide and the daughter kinetochores move towards the opposite poles. In meiosis, on the other hand, the kinetochores of each bivalent are undivided when they go to the poles; hence, there is segregation of the chromosomes instead of the chromatids. So, the anaphasic group of chromosomes is of the haploid and not diploid group of chromatids. In this way, there is reduction in the number of chromosomes in meiosis-I. When two daughter nuclei which are haploid divide by the process basically similar to mitosis, the process of meiosis is completed. It is important to bear in mind that meiosis-II is different from mitosis in respect of three points: (i) The number of chromosomes is half. (ii) Generally, chromatids are very much separated from each other and do not show relational coiling, (iii) Each chromatid may be genetically different at the end as compared with the beginning of meiosis. Meiosis occurs in the life of an individual because of the event of syngamy which is to take place later when nuclei of two gamete cells unite. Therefore the purity of gametes is maintained through the second meiosis. It will be, therefore, seen that the diploid stage is changed to the haploid stage through meiosis and the former stage is again brought back through syngamy. part of a supply of the comment of the spine of the same

each other and the homelingues are ready to appreciate in respinsing the therefore chromatics of each homological chromosome, bound by their consectors. Seem to more constructable respective poles, to telephases I chromosomes inche strendy regraded

#### SUMMARY

1. In an unicellular organism, cell division is a means of reproduction. The growth and development of an organism depends on the growth and multiplication of its cells. Several scientists limit the term growth to this process of cell division. The terms karyokinesis and mitosis only refer to nuclear division. The term cytokinesis is used for the division of cytoplasm. Cytologists have divided the process of cell division into five stages; interphase, prophase, metaphase, anaphase and telophase.

#### Mitosis

2. Interphase is the time interval between the end of telophase and commencement of the next prophase. It is divided into three sub-stages: G1, S and G2 on the basis of synthetic activities. During G1 chromosomes are generally observed as single-stranded structure while in G2 they become double-stranded, possessing two chromatids each, because of DNA duplication during S stage. Very significant changes occur in the nucleus as well as the cytoplasm during prophase. There is condensation of chromosomes and this process continues throughout the successive stages. In animal cells, the spindle develops in the cytoplasm and asters move apart in late prophase. There is also disintegration of the nuclear membrane. Metaphase begins when the chromosomes reach the plane of the equator and arrange themselves radially at the periphery of the spindle. The kinetochores which had held together two chromatids of the chromosome divide and the resulting kinetochores move apart, chromatids get separated and their movement towards respective poles starts. In anaphase, daughter chromosomes (chromatids) of each group start migrating to their respective poles. In telophase, chromosomes become less compact and the chromonemata coils unwind. The nuclear membrane is formed around each nucleus and nucleoli appear at the site of nucleolar organisers or satellites. Cytokinesis takes place by constriction of cytoplasm in the equatorial region which extends deeper and deeper resulting in division of the cell. In plant cells, there is formation of phragmoplast and the cell plate for dividing the cell.

#### Meiosis

3. Meiosis refers to the reduction division resulting in the formation of gametes. In the diplotene of prophase-I, chromosomes are seen as long and uncoiled filaments. The replication of DNA and the protein histone coincides with the doubling of chromosomes. Each pair of homologous chromosomes constitutes a bivalent. In the zygotene substage, homologous chromosomes pair. At pachytene, crossing over takes place involving the exchange of genes between certain segments of homologous chromosomes. The tendency of repelling of homologues in a bivalent becomes apparent during diplotene and chiasmata appear. During diakinesis, the contraction of chromosomes is nearly maximum and the chromosome pairs are nicely spread throughout the cell. The terminalisation is almost complete. In metaphase-I, two members of each homologous pair arrange at the equator of the spindle. The kinetochores of the bivalents repulse each other and the homologous are ready to separate. In anaphase-I, the daughter chromatids of each homologous chromosome, bound by their kinetochores, begin to move towards their respective poles. In telophase-I, chromosomes have already reached

their respective poles and the stage ends in the formation of daughter nuclei known as spermatocytes in males and oocyte-II and the first polar body in females in the case of animals. In the case of plants, they are called dyads which will give rise to microspore cells in the male organs and megaspores in the female organs in meiosis-II. The synaptonemal complex is present between the homologues of a bivalent. It comprises a dense central element on either side of which there is a dense lateral element. According to Meyer, the synaptonemal complex takes part in chiasma formation and crossing over.

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# 14. Cytological Techniques

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It is essential for a proper understanding of cytological techniques to be conversant with the basic principles and methods of general biological microtechniques. Cytological microtechnical procedures require careful handling of materials. It must be borne in mind that no two species react similarly in respect of identical technical schedules. Therefore, it is necessary to know the composition of killing and fixing fluids and the staining schedules that are suitable for a particular plant or animal species. It is, only through experience and proper multiplication of stains and staining procedures that one can learn how to get optimum optical contrast.

As the present book is theoretical, only a broad outline of cytological technique is given here. It only gives the schedules of fixative and staining that are usually used in practical classes. A selected list of books dealing with cytological techniques is given at the end of the chapter for further reading.

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Levine L. (1931), The Call in the state of the Section Vol.

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#### Apparatus

The following are usually needed in a practical class:

- 1. Microscope A student or medical microscope with an oil immersion lens may be used. The student must get acquainted with all the parts of the microscope and its manipulation.
- 2. Microtome This will be required at a later stage. There are two types usually in use; the sliding and the rotary or rocking type. Both types require careful handling. The rotary or rocking type microtome is only used for examining sectioning materials embedded in paraffin.

It is advisable that a student use his own microtome knife since laboratory knives are usually not sharp because of careless handling by students. Proper sharpening of the knife from time to time ensures better sectioning of the embedded material. Knives should be sharpened on a hone or leather strap that is stiff or mounted on a wooden block. If microtome knives cannot be afforded, safety razor blades can be used. Thin blades should be avoided. The blade can be fixed on the microtome knife with the help of melted paraffin.

- 3. Paraffin embedding For this, an electrical oven with a thermostat is preferable. The temperature range must be between room temperature and 70 or 100°C.
- 4. Staining jars and boxes Coplin jars which can hold five slides vertically and horizontal boxes which can hold 10 slides are required for staining sections of microtomed embedded material.
  - 5. Slides Belgian glass slides with smooth edges and a uniform thickness of 1 mm are

on county of remains and a clare of classes.

preferable. Greenish slides can be used, as they are less likely to corrode. The standard size

- 6. Coverslips No. 1 coverslips (0.17 mm thickness) of Japanese make are quite suitable for cytological work. For smears and squashes, square coverslips (18 mm) are suitable. Rectangular ones of various sizes can be used for slides with serial microtomed sections. They are 22 mm in breadth and 30 ... 70 mm in length. No 0 coverslips can be used while working with an oil immersion lens.
  - 7. Petri dishes These can range from 2.5 to 10 cm in diameter.

# Reagents

- , storer a more at the come of the older standard adheaves still in common use. 1. Dehydrating reagents For dehydration of tissues, reagents possessing hygroscopic properties must be used. A dehydrating reagent must be a liquid mixing equally well with water, ethyl alcohol, balsam and paraffin. It should not cause desiccation of the tissues.
- 2. Ethyl alcohol It is also referred to as alcohol or ethanol. Absolute alcohol is of 100% concentration while rectified spirit is of 95% concentration. Till recently, it was the most common dehydrating reagent, but as it is suspected of causing shunkage and hardening of tissues, other fluids are now being preferred in its place. It is, however, quite safe to use it up to 50% and then transfer the material into some other suitable fluid.
  - 3. Iso-propyl alcohol It does not harden materials as much as ethyl alcohol and is a satisfactory dehydrating agent. Do not, however, substitute it for ethyl alcohol in killing fluids.
  - 4. Normal butyl alcohol It is soluble in water up to 8% by volume. It causes slight shrinkage of many tissues and often shows a tendency towards hardening materials.
- 5. Normal propyl alcohol According to Johansen (1940), animal tissues can be directly transferred from water into paraffin through three changes of this reagent. Besides it does not harden or shrink animal tissue. It has, however, not received sufficient attention in the case of plant tissues.
  - 6. Tertiary butyl alcohol It is the best dehydrating reagent for the beginner and is also probably the safest (Johansen, 1940). It mixes with all reagents commonly used in the laboratory and gives the best results.

# involved and playedy poor the role with an earliest allow it to cook Boladye the many

Majding collient serious attendent ing the juliant Most of the fluids used as clearing reagents are not miscible with water. Tissues are, therefore, to be dehydrated completely before using a clearing regent. Some of the common clearing reagents are as follows.

- 1. Xylol It is also called xylene. After dehydration, a very close series of xylol and absolute alcohol is required before transferring the material from xylol to paraffin, to avoid crystallisation of the material. Remove every trace of xylol before embedding it in paraffin. Do not leave tissues in xylol for too long or else they may become extremely hardened.
- 2. Cedar oil It is a rather slow clearing reagent. It is harmful for the material to be left in cedar oil for too long. For immersion lenses, a different type of cedar oil is available.

#### 150 Cytology and Genetics

- 3. Clove oil It is commonly used for clearing sections of plant tissue before mounting in balsam. It contains about 82% eugenol. To prevent stains from fading, remove all traces of clove oil by washing the slide in xylol. Clove oil makes tissues brittle if left in it for too long.
- 4. Benzol, toluol These can be substituted for xylol but one has to be very cautious when using them since they are of an explosive character.
- 5. Trichloroethylene According to Johanson (1940), this is an excellent substitute for xylol. Paraffin dissolves completely in it.

#### Adhesives

- 1. Mayer's adhesive This is one of the older standard adhesives still in common use. It is prepared by adding to the white of a fresh egg an equal quantity of cent per cent glycerine and 1g of sodium salicylate or crushed crystals of thymol. After shaking it thoroughly, the mixture is filtered through sterile cotton or sterile muslin cloth of two or three thicknesses.
- 2. Haupt's adhesive This is an excellent adhesive for affixing sections in the paraffin ribbon to the slide. It is prepared by dissolving finely ground 1g gelatin, either plain or pure, in 100 ml distilled water at room temperature and then adding to this 2g of phenol crystals and 15 ml of 100% glycerine. After stirring, filter the solution. Smear a drop of this adhesive evenly and thinly on the slide and then immediately add to it 3 to 4% aqueous solution of formalin. This is to help the sections float in the paraffin ribbon.

# **Embedding Media**

1. Paraffin wax Most of the paraffin available in the market is not of good quality as it crystallises readily when cooled, in spite of taking all necessary precautions. It is, therefore, better to get a guaranteed product of a reputed make. The paraffin usually used under tropical conditions has a melting point of 50 to 58°C.

To improve the consistency of paraffin and thus make microtoming easier, mix rubber with paraffin (Johansen, 1940). Heat the wax in a container till it is very hot but not smoking. Then adjust the heat to keep the temperature constant. Add small pieces of rubber to this in small quantities at a time. Stir the mixture, from time to time. When the rubber has melted completely, pour the mixture into a tin can and allow it to cool. Remove the mass after cutting away the tin. For 100 g of paraffin wax 20 g of rubber is required.

2. Celloidin, collosin, parlodion These are highly inflammable forms in nitrocellulose. They are, however, non-explosive. They are available either as tablets or shreds, the latter are stored in water. Synthetic polymers are good substitutes of paraffin wax.

# **Mounting Media**

1. Canada balsam To melt canada balsam do not heat it but dissolve it in xylol or benzon. Neutral and filtered canada balsam in the liquid form is also obtained from the market.

2. Euparal It consists of camsal, sandarac, eucalyptol and paraldehyde. It has a much higher refractive index than canada balsam. As euparal intensifies haematoxylin stains it is often used in the case of micropreparations stained with haematoxylin. Mounting with euparal is done from 95% alcohol.

3. De Pex This is fast becoming a popular mounting media.

4. Glycerine jelly Prepare this by dissolving one part by weight of gelatin of a good quality in six parts by weight of distilled water for two or more hours. Add seven parts of glycerine to this and finally 1 g phenol crystals to each 100 g. Warm the mixture for about 15 min., with continuous stirring, till the flakes produced after addition of phenol disappear completely. Filter it while it is still warm, through two or three thicknesses of muslin cloth, into a suitable bottle. It solidifies on cooling. For remelting, it is preferable to cut it into small parts and then melt them.

#### Pretreatment

To bring out a good separation of chromosomes, for clearing cytoplasm and separating the middle lamella, pretreatment of the material is sometimes necessary before fixation, especially for the study of mitosis and meiosis. Sometimes, pretreatment by removing undesirable deposits on the tissue is done to help in the rapid penetration of the fixation.

# Methods of Separating Chromosomes

1. Pretreat the material at 15 to 20°C with 0.015 to 0.02% aqueous solution of colchicine for 30 min to 1 h.

2. Pretreat the material with an aqueous solution of 8-hydroxyquinoline (Tjio and Levan,

1950) of 0.008 M (0.29 g/l) concentration for 3 h.

3. Pretreat the material with saturated aqueous solution of p-dichlorobenzene at 15°C for 3 h.

## Methods of Clearing Cytoplasm

1. Pretreat the material with 1 N HCl for a short while. After this, wash it thoroughly so that no excess acid-soluble materials remain. Since the basophila of chromosomes are affected by acids, mordanting may be required after pretreatment and washing.

2. Pretreat the material with ribonuclease enzyme of 100 ppm concentration for about an hour. Ribonuclease enzyme digests RNA, which is the main constituent of cytoplasm.

3. When the oil content in the cytoplasm is heavy, pretreat it with dilute alkali solution in NaOH or NaHCO3. It saponifies the oil and thus removes it. Wash the material thoroughly in water.

# Methods of Removing Deposits

1. Use hydrofluoric acid to remove siliceous deposits.

2. Remove oily or other secretory deposits on cell walls by using Cornoy's fluid with chloroform.

# Killing and Fixation

In cytology, the term 'killing' implies the sudden termination of the life process of individual cells which constitute the tissues or the whole organism. The reagent used for killing must penetrate every cell in the organism or pieces of tissue cut or else the killing is not complete. Killing always precedes fixation because the reagents used for killing penetrate tissues faster than those employed for fixation. Alcohol is the primary killing reagent.

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Fixation involves the preservation of all cellular and structural elements to retain a reasonable facsimile of their appearance when alive. A good fixative alters the cell chemistry least and preserves the cell structure best (Schiller, 1930). Rapid penetration of the fixative is necessary for its action to be uniform. Fixatives such as Atten's Bouin or acetic acid can be administered warm (about 35 to 40°C), to expedite the process of penetration. It also increases effectively coagulation and hardening. When the reagents used in a fixative penetrate the cells, the colloidal system of the protoplasm undergoes a change of state which is irreversible. The ultimate object of fixing protoplasm is to make the invisible or barely visible structures easily observable (Johansen, 1940). As the staining of tissues is only another process of increasing the optical differences, it ultimately depends upon the nature of the fixed protoplasm.

Chromosomes are thread-like structures made of fibrous protein and are the most resistant materials in the cell (Darlington and La Cour, 1976). They are covered with a sheath of nucleic acid which causes all chromosomes to stain with the same basic days. Acetic acid swells them a little while chromic and osmic acids cause their contraction. However, none of these reagents bring about displacements.

Healthy material must be chosen for fixation. For the study of mitosis choose root tips taken from plotted plants or plants grown on a soil ball which is not very wet or very dry. They can also be taken from a water culture. For the study of meiosis use the testes of immature or young animals.

Since animal cells have no cellulose walls, their tissues become fixed more rapidly than those of plants. However, in both kinds of tissues, the process of hardening takes several hours.

All the disadvantages that are experienced in the usual methods of fixing can be eliminated by fixing through freezing at low temperature (Sharma and Sharma, 1972). In this method, cooling must be rapid so that the water does not get time to crystallise. Water from the material is frozen into amorphous ice in a cooling bath at a temperature of  $-175^{\circ}$ C produced by condensation of liquid nitrogen. Removal of water and drying are done in a vacuum at low temperature.

Another method of fixing through freezing is to first rapidly freeze it and then dehydrate it at a very low temperature by using n-proponol/n-butanol/ethanol/methanol. When dehydration is complete, the material is brought back to room temperature.

#### Schedules for Some Common Fixatives

Cornoy's Fluid I

Glacial acetic acid Absolute ethyl alcohol 1 part
3 parts

Machous of Jersoving Consolidation

minim trioxide a mydride are 10 rate 1 g and 1 c respectively

10" aque outin auto upa . "01 Absolute ethal alcohol 9.5% acrecous chrosule gold

in the modification Bi5 (Sharma and Sharma-

Use it in case of all plant, animal and human materials. The period of fixation is 15 min to 24 h at cold or room temperature. Wash the fluid with 70% alcohol and store the material full at most veltow count can be camoued. This facility is community in 70% alcohol. unimal disease. In the model after hit the proportions of glacus acetic seid turn snow

#### Cornov's Fluid II

| Glacial acetic acid    | bad part |
|------------------------|----------|
| Chloroform             | 3 parts  |
| Absolute ethyl alcohol | 6 parts  |

The rest as above.

# Navashin's Fluid

SOLUTION A

Stoolyour row 2 ad 3

| Chromic anhydride   | of Ad payability | 1.5 g |
|---------------------|------------------|-------|
| Glacial acetic acid |                  | 10 ml |
| Distilled water     |                  | 90 ml |

SOLUTION B

| 40% aqueous formaldehyde | 40 ml |
|--------------------------|-------|
| Distilled water          | 60 ml |

Immediately prior to use, mix the two solutions in equal parts. The period of fixation is 24 h. After fixation, wash the material in running-water for 3 h. This fixation is good for floral buds and root tips. The bib second of a day with the second of th

#### Randolph's (CRAF) Modification

#### SOLUTION A

| Chromic anhydride   | 1 g  |
|---------------------|--|
| Glacial acetic acid | no more but minores snoups of  |
| Distilled water     | 92 ml hos observers  |
| Yes on the last     | The House of the Party of the P |

SOLUTION B

| 40% aqueous formaldehyde | 30 ml         |
|--------------------------|---------------|
| Distilled water          | 70 ml         |
| Distilled Water          | THE LABOUR DE |

To be used in the same way as Navashin's fluid. Washing is to be done in 70% alcohol. The period of fixation is 10 min to overnight.

# Bouin's Fixative (Original)

| Saturated aqueous picric acid | 75 ml |
|-------------------------------|-------|
| 40% aqueous formaldehyde      | 25 ml |
| Glacial acetic acid           | 5 ml  |

Dissolve pieric acid in distilled water and keep it as a mixture with formalin and acetic acid. Just before use, heat the mixture at 37°C and add crystals of chromic anhydride. Stir well

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and then add urea. Keep the material in the fixative at 37-39°C and cool the mixture gradually. The period of fixation is 9 to 12 h. Wash the material thoroughly in 70% alcohol frequently till no more yellow colour can be extracted. This fixative is commonly used to preserve animal tissues. In the modification B3, the proportions of glacial acetic acid, urea and chromium trioxide anhydride are 10 ml, 1 g and 1 g respectively.

In the modification B15 (Sharma and Sharma, 1972), in addition, 2 g urea and 1.5 g Clacial acetic soid

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chromium trioxide anhydride are used.

#### Chromo-nitric Acid

| 10% aqueous nitric acid   | 4 parts    |
|---------------------------|------------|
| Absolute ethyl alcohol    | 3 parts    |
| 0.5% aqueous chromic acid | The Parish |
| solution                  | 5 parts    |

The period of fixation is 4-5 h followed by washing in 70% absolute alcohol for 2 to 3 TOUR Hall programme and the street days.

## Propionic Acid-alcohol

| Propionic acid    |  | 1 part  |
|-------------------|--|---------|
| 95% ethyl alcohol | The state of the s | 3 parts |

The period of fixation is 24 to 36 h followed by washing with 70% alcohol. This fixative gives good results in case of plant materials with small chromosomes. In one modification (Sharma and Sharma, 1972), 100 ml each of propionic acid and 95% ethyl alcohol and 0.49 of ferric hydroxide are used. Add a few drops of carmine to each 10 ml of the fixative.

#### Osmic Acid Fixatives

## (a) STRONG FLEMMING

| 1% aqueous chromic acid solution | 15 ml          |
|----------------------------------|----------------|
| Glacial acetic acid              | 1 ml           |
| 2% aqueous osmic acid solution   | 4 ml as per    |
| Z/o aqueous osmie and series     | Baker (1950)   |
| 2% aqueous chromic acid solution | 100 ml         |
| 2% osmium tetraoxide solution    | 53 ml          |
| 10% aqueous acetic acid solution | 133 ml as per  |
| 10% aqueous assure and           | Darlington and |
|                                  | La Cour (1960) |

# (b) BENDA'S MODIFICATION OF FLEMMING'S FIXATIVE

| 1% aqueous chromic acid solution | 15 ml   |
|----------------------------------|---------|
| 2% aqueous osmic acid solution   | 4 ml    |
| Glacial acetic acid              | 3 drops |

According to Makino (1934), if this fixative is diluted with an equal part of water it gives good results in case of spermatogonial divisions of teleosts.

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The abovementioned fixatives must be mixed thoroughly before use. For bulk fixation use Strong Flemming. The period of fixation is 1 h to overnight, followed by washing in running water for an hour to overnight. According to McClung (1918), fixation in cold is more effective. These fixatives are used both for plant and animal materials.

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### Embedding and Microtomy

Where hand sections, smears or squashes are not possible, a microtome should be used. For microtomy, the material should be embedded in paraffin or celloidin after fixation.

# Steps in Paraffin Preparation those allege W24 or beauty at barreton orbit motions

- 1. Fix the material to be sectioned. A glass phial or small specimen tube (10 ml) must be used.
  - 2. Wash the material with water with three quick changes in the phial.
- 3. Dehydrate it using distilled water-alcohol mixtures in grades series (30% 3-4 h; 50% 3-4 h; 70% overnight, 80% 3-4 h; 95% 3-4 h) and finally absolute alcohol (left overnight with 3-4 changes). The management of the first and the second and
- 4. Transference and elearing Replace alcohol by xylol/chloroform by using alcoholxylol/chloroform mixture in steps of 25, 50 and 72%, 2 h in each and finally by pure xylol/ chloroform in which the material is cleared. Three or four changes or xylol/chloroform are necessary.
- 5. Infiltration First add wax shavings of paraffin (usually of 56-38°C) to the xylol/ chloroform bit by bit at room temperature till zylol/chloroform becomes saturated with paraffin. Then keep the phials on top of the oven or hot plate at about 30°C for about 2 days, adding more wax at intervals. This wax is replaced by molten wax and phials are placed inside the oven at 60°C for about 4 hours and there should be 2-3 changes of wax so that no trace of xylol/chloroform should be left in the phials.
- 6. Embedding For embedding materials in paraffin, use a small tray of strong and thick paper or a small petri dish smeared inside with glycerine. Transfer the contents in the phial along with some more molten wax to the paper tray or petri dish and arrange properly. When a skim is formed on the wax, immerse the paper tray or petri dish in cold water and take the paraffin block out. order and taken stellary dismissib the stellar and take the paraffin block out.
- 7. Trimming of block and microtomy Cut the part of the block containing the material to be sectioned and trim it properly. Mount it on the holder with wax and microtome it to the desired thickness (usually 8-12 µ). Place the ribbon on the slide smeared with an adhesive and float it in water. For stretching and straightening the ribbon, keep the slide on a hot plate. Drain off the water and allow the slide to dry on the warm plate by keeping it for 4-24 h. The sections are now ready for staining. Add annual and avoices it are now ready for staining. This command is called merday and the process is known as a little annual Tennelly

### mordanting and staining are combined by oversing a single staining continue completion Smears and Squashes and a perform that the same transform of the

in politics gratel total b is built a sus conservation and hollie-In cytological micropreparations, smears and squashes are now gaining preference to sections except in the case of extremely minute masses of materials because the period of

fixation is faster and the entire procedure takes much less time. Moreover, one can examine single layers of large cells in their totality.

#### **Smears**

Smearing is done when the tissue is soft, e.g. anthers and soft testes. Half-anther or a part of soft testes is placed on the slide and pressed gently with a flat-honed scalpel so that there is direct spreading of the cells. The slide is then immediately inverted over a dish containing the fixative. In this method, it is possible to combine fixation with staining with a few exceptions. For instance, it is necessary to fix flower buds in Farmer's fluid (acetic-ethyl alcohol 1:3) before smearing in carmine (Sharma and Sharma, 1972). After fixation, the material is placed in 45% acetic acid for 15 to 30 min so as to counteract the effect of ethyl alcohol and to soften the tissue.

#### Squashes

Belling (1926) devised the standard iron-acetocarmine method for studying meiosis in pollen mother cells (PMCs) but it is now also extended widely to animal tissues (Darlington and La Cour, 1976). The tissue is teased out in a drop of acetocarmine with an unplated iron needle and then mounted directly under a coverslip. The iron acetate formed serves as a mordant for the carmine which differentiates chromosomes and cytoplasm.

In case of certain tissues or root tips maceration, the material is placed in N HCl for 5-10 min at 60°C and then fixed. For pollen grains, the most suitable maceration method is to immerse the material in equal parts of concentrated HCl and 95% alcohol 5 min without warming. It is then hardened in Cornoy's fluid for 10 min before staining.

### Staining the oven at 60% for about 4 hours and there should be 2.2 can gnining

Staining is a process of adsorption (Baker, 1950). It may be simultaneous, i.e. both chemical and physical (Sharma and Sharma 1972). For the microscopic study of any tissue, it is subjected to various processes such as killing, fixation, dehydration, clearing, embedding, etc. After undergoing such treatments, tissues usually do not retain enough colour to make their constituents distinctly visible under the microscope. Hence, it is necessary to use certain dyes for colouring the tissues. This is called *staining*.

Sometimes it is necessary to use two or three kinds of stains for distinguishing certain tissues. This is known as differential staining. In such cases, components of the tissues can be differentiated because of their property to retain dyes of contrasting colours.

The dye generally adheres to the cytoplasm directly but it is rarely so in the case of the nucleus. Therefore, for staining the nucleus it is first treated with another compound. This compound is called *mordant* and the process is known as *mordanting*. Generally, mordanting and staining are combined by preparing a single staining solution containing both the mordant and the dye. This method is known as *indirect staining*. As in this method, the entire tissue gets stained, a differentiating solution (usually a weak acid) is used. When the tissue is transferred to this solution, the stain taken by the cytoplasm is removed and only the nucleus remains coloured.

### Types of Stains

Stains are classified into two principal types: (i) natural and (ii) synthetic. The former dyes are obtained from plant or animal sources, while the latter are generally artificial chemical compounds consisting of an acid and a base depending on the colouring of the acid or the base. Artificial stains are of three types: (i) acid, (ii) basic, and (iii) natural. In the case of acid stains, only the acid component becomes coloured, whereas the base remains unstained, e.g. only the cytoplasm is stained. As regards basic stains, the reverse happens, i.e. only the nucleus gets stained. This is owing to the interchange of ions when the sodium salt of an acid dye and the chloride of a basic dye are mixed, e.g. Romanovsky stains.

## Common Dyes Used in Cytological Techniques

### Haematoxylin

This is a natural dye derived from the wood of Haematoxylin compechianum L. of the Caesalpiniaceae. Since the dye solution has very little or no affinity for tissues, it is necessary to use a mordant, e.g. iron or aluminium alum.

### Heidenhain's Haematoxylin

This is 0.5% solution in distilled water. It is, however, better to have a 10% solution in absolute alcohol and then dilute it into the required part with distilled water to 0.5% (Johansen, 1940). In order to ripen the solution, it is placed in a shallow, wide evaporating basin and exposed to ultraviolet rays for about 45 min, their source being a powerful quartz mercury-vapour lamp kept at a distance of 60 cm. The solution is to be stirred frequently during exposure.

The present author prefers to prepare a 1% solution in boiling distilled water and continue boiling for about 10-15 min. Then it is allowed to cool. A drop of the solution is then added to a beaker containing tap-water. When the colour of the water becomes faintly violet, the solution is ready for use. It can be preserved for a week.

### Delafield's Haematoxylin

Neumal red. It is a weakly beaut and writes and is reced in A solution of 4 g of haematoxylin is prepared in 25 ml of 95% alcohol. This is then added drop by drop to 400 ml of a saturated aqueous solution of ammonium aluminium sulphate. The solution is exposed to light and air for four days. Ten ml of pure glycerine and 100 ml of methyl alcohol are added and it is allowed to stand exposed to air for about two months. When the colour of the solution becomes darkened, it is ready for use. Alternatively a mercury vapour lamp can be used in which case it takes only two hours.

### Ehrlich's Haematoxylin

There are the stage Haematoxylin with the babbe of the leg more with the stage of the leg man with the our many hours are Glacial acetic acid of same weeks 5 ml worth abstantion

Cooressive and Magressive Steining

A. Meshyleng blue. It is a heaterfully good warm an

| Glycerine             | 50 ml |
|-----------------------|-------|
| Absolute alcohol      | 50 ml |
| Distilled water       | 50 ml |
| Potoch alum in excess |       |

exid on the base. Actificial states, are of three expositional fitty man, and only when

Tyres of Scains

dves are obtained in

### Harris' Haematoxylin Boling Ambared Manurana Assault Visio, select Dion to same off all

This is excellent for plant materials.

| Haematoxylin                | 5 g    |
|-----------------------------|--------|
| Aluminium ammonium sulphate | 3 g    |
| 50% alcohol                 | 100 ml |

The dye and ammonium alum are dissolved with the aid of heat and 6 to 7g of mercuric oxide (red) are added and the mixture heated for about half an hour. It is then filtered and made up to the original volume with 50% alcohol. It is acidified by adding 10 drops of HCl.

#### Carmine

This is a bright red natural dye. It is the lake obtained by adding alum to cochineal. The latter is a yellowish red powder prepared by grinding dried bodies of cochineal insects and extracting the colouring matter. The essential colouring agent in this powder is carminic acid.

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### Coal-tar Dyes

1. Acid fuchsin (Triamino triphenyl methane-rosanilin It is a complex mixture of sulfonate derivatives of basic fuchsin. It stains with parenchyma, cortex and cellulose walls. It can be used as a mitochondrial stain.

2. Basic fuchsin (pararosanilin) This belongs to the basic triamino triphenyl methane group. It is easily soluble in water and alcohol and is used in preparing Feulgen stain.

3. Crystal violet (Gentian violet) This is a member of the basic triamino triphenyl methane group. It can be used in 1% distilled water solution. It is usually kept in a dropping bottle.

4. Methylene blue It is a bacteriological stain and belongs to the basic thiazine group.

5. Neutral red It is a weakly basic azin series and is used in vital staining.

6. Vital red (Brilliant Congo red) This belongs to the acid azo group and is an important vital stain.

7. Picric acid A strong acid, it belongs to the nitro group. It is always stored in water and is used as a differentiator for haematoxylin, safranin and crystal violet. It is a general cytoplasmic stain.

8. Safranin It is a nuclear stain belonging to the basic azin (safranin) group.

### Progressive and Regressive Staining

In progressive staining, the stain is added to the tissue till the desired depth of the colour is obtained. However, in regressive staining, the sections are first overstained and then the

Stops I and 2 as A. usene leucobasic fluctusin.

excess stain is removed by transferring the sections in a differentiating solution. As already mentioned, nuclei possess the property of holding more dye than the cytoplasm. So when it is observed that sufficient dye has been removed from the cytoplasm and the correct depth of stain in the nuclei is obtained, the sections are washed thoroughly in running Pass time covered p and the slide, if required. through 80% atomic to a covered pass time covered pass absolute alcohol with two enanges. I min each.

8. Place a drop of suparal and recombine the slide and coverstip so that mounting is done,

### Staining Methods B. Feulgen Methon for Backons and Smears (Dollington and La Cour.

### Feulgen Reaction

This is regarded as the most effective method of chromosome staining. Feulgen and Rossenbeck devised a method on the basis of Schiff's reaction for aldehyde which causes specific staining of the nucleic acid of chromosomes. The chemical basis of the reaction involves two main steps:

1. When hydrolysis with normal HCl takes place, the purine-containing DNA fraction is separated from sugar, with the result that the aldehyde group of the latter is exposed.

2. The reactive aldehyde group combines with fuchsin sulphurous acid and a typical magenta colour is obtained. When the base is removed, the carbon atom of the furanose sugar is arranged in such a manner that a potential aldehyde capable of reacting with fuchsin sulphurous acid is formed. This ribose sugar with an -OH in place of -H at carbon 2 does not get hydrolysed by N HCl. Hence, it does not react with fuchsin sulphu-& Leave in tapwater for 10 min with two changes. rous acid.

### Dissect endosporm on the stide in a drop of 45% acetic acid on the stide placed on Preparation of Feulgen Stain (Leucobasic Fuchsin)

Dissolve 0.5 g of basic fuchsin in 100 ml of boiling distilled water and cool the solution to 58°C. Then filter it and bring it down to the room temperature. Add 10 ml of N HCl and 0.5 g of potassium metabisulphite to this and pour it into a flask and seal it with an airtight stopper. Wrap the flask in black paper and keep it in a cool dark chamber. Take it out after 24 h. If the solution is translucent and of straw colour, it is ready for staining. If not, then add 0.5 g of animal charcoal powder to it and shake the flask thoroughly. Keep it back in a dark chamber overnight. Filter and use it. Securing bacarates vilo (any preparative as described

### A. Feulgen Squash Method (Darlington and La Cour. 1976)

This method can be used for all tissues except endosperm.

- 1. After fixing the material for 2 to 24 h, rinse it with two to three changes of water.
- 2. For maceration, hydrolyse it in N HCl at 60°C for 6-10 min or more and stain in leucobasic fuchsin (plant tissues 2-3 h, animal 1-2 h).
- 3. Place the material in a drop of 45% acetic acid on a slide and tease out small pieces of tissue with the help of an arrow needle or the end of a scalpel so that small groups of cells are obtained.
- 4. Film the coverslip and put it in position. Wrap it in folds of filter or blotting paper and apply pressure with the blunt end of a thick pencil with precaution so that the coverslip is not broken and there is no sideway movement of it. I the transfer of the state of the st

5. Pass the slide over a spirit flame 4 to 6 times, taking care that the contents do not mentioned, nuclei possess the property of holding more due ragnitude or unlasm. belief the

6. Turn the slide face downwards in a smearing dish containing 40% alcohol so that the

coverslip will separate from the slide after 3 to 10 min.

7. Pass the coverslip and the slide, if required, through 80% alcohol for 2 min, and absolute alcohol with two changes, 2 min each.

8. Place a drop of euparal and recombine the slide and coverslip so that mounting is done.

Staining Methods

### B. Feulgen Method for Sections and Smears (Darlington and La Cour, 1976)

Steps 1 and 2 as A, using leucobasic fuchsin.

3. Give three changes of 10 min each of freshly prepared SO<sub>2</sub> water.

- 4. Rinse first with distilled water and then in each of the alcohol series 20, 60 and 80%.
- 5. Keep in absolute alcohol for 2-3 min.

6. Mount in euparal.

### C. Feulgen Squash Method for Endosperm (Darlington and La Cour, 1976)

- 1. Fix developing seeds in acetic alcohol (1:3) and harden in 95% alcohol overnight.
- 2. Place in 70, 50 and 30% alcohol, 30 min each.
- 3. Rinse in water for 10 min.
- 4. Hydrolyse in N HCl at 60°C for 8 to 12 min.
- 5. Stain in leuco-basic fuchsin for 2 h.
- 6. Leave in tapwater for 10 min with two changes.
- 7. Dissect endosperm on the slide in a drop of 45% acetic acid on the slide placed on the dissecting microscope with the help of a tungsten needle pointed in molten NaNO2. For rest of the steps follow 4-6 in A.

### D. Haematoxylin Method

It is useful for any smear or section.

- 1. After rinsing in distilled water, mordant in 4% iron alum for 10 to 30 min.
- 2. Rinse in running water for 10 to 20 min.
- 3. Stain in haematoxylin (any preparation as described before) for 5 to 15 min.
- 4. Rinse in water and destain in saturated aqueous picrid acid for 5 to 20 min. or 4% 1986年中的1980年中国的1986年中国 iron alum for 3 to 10 min.
  - 5. Add one or two drops of ammonia water so that the stain turns blue.
  - 6. Rinse in running water for 20 to 30 min.
  - 7. Pass quickly through 30%, 60%, 90% and absolute alcohol.
  - 8. Examine in clove oil.
  - 9. Place in xylol and mount in canada balsam. pieces of classic with the help of an arrow needle or the end of a scalpel so that

### E. Belling's Iron Acetocarmine Method for Smears

1. Smear the tissue on the slide under the coverslip in the acetocarmine stain (to which a dash of ferric hydrate has been added). The manufacture of the second has been added to the second has

- 2. Pass over a spirit flame 4 to 5 times but do not let it come to a boil.
- 3. Keep the preparation in a petri dish containing 10% acetic acid.
- 4. When the coverslip gets separated from the slide, place both of them in a 1:1 mixture of absolute alcohol and acetic acid.
- 5. Pass through mixtures of 3:1 and 9:1 alcohol-acetic acid and then to absolute alcohol, and finally place it in xylol.
- 6. Recombine the slide and coverslip and mount in canada balsam. Acetocarmine can be replaced by propionic carmine (when propionic acid is used in a fixative) or aceto-orcein stains. Acetic orcein gives very good results in case of many root tips. up of chromosomes, rapidly growing but budy out he used. Year young to the or

- Blood Smears and the state of t 1. Take a fresh drop of blood on a clean dry slide and spread it thinly with the aid of another slide. A safety control of the corporate of supplication of guidens as a safety
- 2. Stain in one or more drops of Wright's stain [Wright's stain powder 0.1 g and methyl alcohol (neutral) 60 ml to be ripened for about 20 days] for 1 to 2 min.
  - 3. Add equal quantity of water on the slide and allow it to remain for 2 to 3 min.
- 4. Wash with water and gently blot with a filter paper taking care that the smear is not they be a countly in branches of few local at the personal mediation a consider disturbed.

### Quick Freeze Method of Making Permanent Squash Preparations (Conger and Fairchild, 1953)

This is to be used after Feulgen or acetic-orcein.

- 1. Place the back of a slide on a block of dry ice and press it so as to secure a good contact. The root add could be the supplementary to Manual and a School of the supplementary to the supplementary 2. Freeze for 20 s or longer, and the state of the state
- 3. While the slide is still lying on the dry ice, remove the coverslip with the help of a needle. So to the and the covered this or assess a planta such as the language and beauty.
- 4. Place the slide and/or coverslip immediately in 95% alcohol before thawing begins, and keep it there for 5 min. 5. Transfer it to absolute alcohol and leave it there for another 4 to 5 min.
- 6. Mount it in thick euparal and dry for two days.

Please note that most of the material sticks to the slide if no adhesive is used and to the coverslip if it is filmed.

### Crystal Violet-Iodine Method

This can be followed in the cases of sections or smears after aqueous fixation. of the or site of the to the table of the M. E.O. from I stop of

- 2. Pass through 10%, 20%, 30% and 50% alcohol, each 5 to 10 min. 3. Bleach in H<sub>2</sub>O<sub>2</sub> in 60% alcohol (100 ml of 70% alcohol to 10 ml H<sub>2</sub>O<sub>2</sub>) for a few minutes.
  - 4. Bring back to water and stain in aqueous crystal violet for 5 to 6 min.
  - 5. Rinse in a solution of 1% iodine and 20% KI in 70% alcohol.

- 6. Transfer to 85% alcohol and then in absolute alcohol, 3 to 5 min each.
- 7. Differentiate in clove oil for 30 s.
- 8. Place in xylol with three changes, 10 min each.
- 9. Mount in neutral balsam.

When temporary mounts are made, the coverslip should be sealed with molten mixture of paraffin wax and gummastic (1:1).

#### Leaves

When root tips are not available for the study of mitosis or for the determination of somatic number of chromosomes, rapidly growing leaf buds can be used. Very young leaves of these buds are excised and pretreated in 0.05 to 0.2% colchicine for 1 to 2 h in light.

### Giemsa Banding Technique (Darlington and La Cour, 1976)

Of late, the Giemsa banding technique has come into operation for its recognition of heterochromatin. It can be used in case of squashes of plant somatic tissue, animal somatic chromosomes in spread-free cells and meiotic chromosomes in mammalian spermatocytes, scattered well. Giemsa stock solution of improved R66 TT Gurr is usually recommended for this technique. Darlington and La Cour (1976) recommend its dilution X 50 with M/15 Sorenson phosphate buffer pH 6.9.

- 1. Pretreat with colchicine, etc. as usual.
- 2. Fix in acetic-alcohol (1:3).
- 3. For separation of cells of plant tissues, treat in 45% acetic acid: 1 N HCl for 15 to 30 min. Heat gently over a spirit lamp till the solution steams, and leave it for a minute.
- 4. Place a drop of 45% acetic acid and macerate the tissue. Place the coverslip over the macerated tissue and pass the slide over a spirit flame once or twice and then press gently from above.
  - 5. Spread free animal cells on the slide by drying in air. Leave it for 15 h or more.
- 6. Remove the coverslip from the slide carefully in case of squash preparations made as per schedule for quick freeze method. After removal of the coverslip allow the slides to dry in air for 1 to 2 days.
- 7. In the case of spread animals, transfer slides in 0.2 N HCl for 1 h at room temperature (20 to 25°C) and then rinse. In the case of plant cells, this step is to be omitted.
- 8. Keep slides in a coplin jar containing a freshly prepared 5% aqueous solution of barium hydroxide at 50 to 55°C (in the case of mouse and human chromosomes) or a saturated solution at room temperature (in the case of plants) for 2 to 20 min.
  - 9. Rinse with distilled/de-ionised water with 5 to 6 changes to remove the scum.
- 10. Incubate in 2 × SS c (0.3 M sodium chloride containing 0.03 M trisodium citrate adjusted to pH 7 with 0.1 M citric acid) at 60°C for 30 min to 2 h.
- 11. Rinse in distilled/de-ionised water quickly and stain in dilute Giemsa stock solution for 11 to 16 h.
  - 12. Rinse quickly in distilled/de-ionised water. Blot with care and allow to dry in air.
  - 13. Rinse quickly in xylol and mount in De Pex or a similar adhesive.

### **Vital Staining**

Non-toxic dyes such as neutral red and methylene blue are used for staining living tissues which can be studied without being killed. Methylene blue has been found to be effective in demonstrating cell division in tissue culture (Sharma and Sharma, 1972). One per cent aqueous solution of this dye or neutral red is usually used for staining. Darlington and La Cour (1976) are however, of the view that vital staining of chromosomes by methylene blue injures the chromosomes.

### **Special Staining Techniques**

#### Mitochondria

- 1. Fix the tissue in 10% formalin at room temperature for 24 h.
- 2. Wash in running water for 4 to 6 h.
- 3. Dehydrate, clear and embed in paraffin.

4. Cut sections on the microtome of 2 to 6 μ thickness.

- 5. De-paraffinize in xylol and pass through absolute alcohol, 90%, 70%, 50% and 30% alcohol in acid fuchsin stain (acid fuchsin 0.2 g, methyl blue 0.1 g and N HCl 100 ml) for 5 min.
  - 6. Wash carefully in distilled water.

7. Dehydrate quickly through 30%, 50%, 70%, 95% and absolute alcohol.

8. Clear in xylol and mount in canada balsam or De Pex. Mitochondria are stained purple red with blue membrane and nucleoli brilliant red.

### Golgi Complex

1. Fix in 2% silver nitrate solution in formalin (2 g silver nitrate in 100 ml of 15% formalin) for 2 to 3 h. Wash quickly in water. Develop in 2% hydroquinone in formalin (2 g hydroquinone in 15% formalin) for 2h. Transfer to 10% formalin and keep overnight for complete fixation.

2. Wash, dehydrate and embed by the usual procedure.

3. Section the tissue paraffin block on a microtone at 6 to 8  $\mu$ .

4. De-paraffinize and clear in xylol.

5. Mount in canada balsam or De Pex.

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# 15. Mendel and his Experiments

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Gregor Mendel, an Austrian monk, was the founder of the modern science of heredity. He is, therefore, often referred to as the father of modern genetics. Mendel evolved an entirely new approach to the concept of the transmission of characters.

Mendel was born on July 22, 1822, in a village called Heinzendorf. His father was an Austro-Silesian poor farmer. His education began in an upper elementary school in Leopnik, about 22 km away from his village. His progress in school was very good, and in 1834 he was sent to the Trophan High School. While studying here, he did private tuition to pay for his expenses. In 1840, he completed graduation with a good record and with some financial help and a little bit of money which he had managed to save, he joined the Philosophical Institute at Olmitz. In 1843, he entered the Augustinian Monastery at Brunn in Moravia (now Brno in Czechoslovakia). He also taught at a local secondary school. In 1850, he joined the University of Vienna but unfortunately failed to get through. In 1854, he joined the Brunn Modern School as a supply teacher and taught natural history.



Gregor Mendel

In 1859, Darwin's Origin of Species was published, which revolutionised biological thought. It also had a tremendous impact on Mendel.

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When Mendel started his experiments on the inheritance of characters in 1857, he had a clear concept about his task of finding out how definite and fixed varieties occurring within a species are related to one another. He knew the best chance of success was in selecting a proper plant so that the problem could be tackled without any complication. He found the desired plant in the garden pea (Pisum satirum). He carried out experiments for 8 years and published the results in a brief paper presented in the Proceedings of the Natural History. Society of Brunn. However, for 35 years, his paper remained unknown. There are three main reasons for this: (i) Biologists were much attracted towards Darwin's theory of evolution and concentrated on the study of the life histories of various plants and animals and tracing their relationships. In fact, the effect of Darwin's book was to divert attention from the manner in which species were presumed to originate. (ii) Biologists never imagined that mathematics had any connection with biology, and so Mendel's statistical approach was beyond their comprehension. (iii) He was far ahead of his times. Consequently, it was not possible to have a proper assessment of his work.

Mendel's work was simultaneously rediscovered in 1900 by de Vries (Holland), Correns (Germany) and Tschermak (Austria). Since they were working on experimental breeding, the significance of their discovery became at once apparent to them. They were able to confirm Mendel's results in pea and other plants. Bateson (England) was the first to show their

application to animals. Mendel's success lay in choosing a material such as the common pea, which is a selfpollinating plant with many varieties breeding true to type and possessing distinct characters, which can be easily seen with the naked eye. He crossed plants possessing sharply contrasting characters. He concentrated on one character at a time and studied its inheritance through several generations. It was only afterwards that he paid attention to two or three characters together. His experimental population was large, which is essential for success since he was dealing with probability.

Besides the common pea, Mendel also worked on beans and Hieracium. But in the latter case, he could not get any indication of the laws which he had found to hold good for the others. It was probably because of this and the fact that his work did not get the due recognition that he gave up research in the later part of his life. Mendel died in 1884 of Bright's (iv) position of the flower (axillery or terminal), by form of the post (inflates, or disease. and (vi) gross colour of the unting god overgettern He obtained the same results in crosses.

### Mendel's Work

Experiments on Pea Margor distroy betakened infrared avode services edites self more Mendel selected two strains of peas, one about 180 cm and other 45 cm in height. He had ascertained previously that each strain bred true to type, i.e. tall plants gave rise to only tall progeny and the dwarf ones to only dwarf progeny. When the crossed these two strains artificially, he observed that it made no difference whether he used tall as the pollen parent or vice versa. In every case, he obtained only talls. The hybrid talls were sometimes slightly taller than the tall parent. This hybrid belonged to what is called the  $F_1$  (filial) generation. He termed tallness as the dominant character and dwarfness as the recessive character. He further noticed that when the hybrids from each cross were self-pollinated, in the subsequent generation there were 787 tall and 277 dwarf plants, which is approximately in the ratio of 3:1 (Fig. 15.1.). This hybrid generation is called the  $F_2$  generation.

When plants of the  $F_2$  generation were self-fertilised, Mendel observed that the dwarf plants only gave rise to dwarf progeny while the tall plants were of two types. About 1/3 of them produced only tall offspring while the rest produced both tall and dwarf progeny in the ratio 3:1, i.e. their behaviour was just like that of the  $F_1$  hybrid (Fig. 15.1).

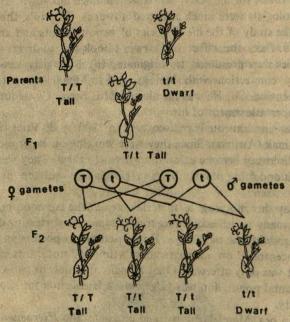


Fig. 15.1 Mendel's experiments on pea, 3: 1 ratio.

Besides height, Mendel dealt with other six characters, namely: (i) colour (red, white) of the flower, (ii) colour of cotyledons (yellow, green), (iii) form of the seed (round or wrinkled), (iv) position of the flower (axillary or terminal), (v) form of the pod (inflated, contracted, and (vi) green colour of the unripe pod over yellow. He obtained the same results in crosses involving any of these pairs of contrasting characters.

#### Laws of Mendel

From the results described above, Mendel formulated certain remarkable hypotheses. These are known as Laws of Mendel.

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#### Law of Dominance

According to this law, hereditary characters are determined by particular units of heredity, called factors or genes. One gene bears one of the contrasting characters, e.g. tallness or dwarfness. Such genes which are alternative in inheritance form a series, each called an allele or allelomorph since it is at the same locus in homologous chromosomes. The character which expresses itself (e.g. tallness) is the dominant and the alternative character which is not expressed is the recessive (e.g. dwarfness).

### Law of Segregation

According to this law, the genes are segregated during the formation of gametes so that only one of the pairs is transmitted by a particular gamete. This is to maintain the purity of gametes, as per Mendel's hypothesis. When a male and female gamete unite and form a zygote, the double number is restored.

Dominant genes are represented by capital letters and the recessive ones by small letters. The crosses between tall and dwarf plants are diagrammed in Fig. 15.2, using these symbols.

Each gamete of a tall female parent carries a gene for tallness T, while each gamete of the male dwarf parent carries a gene for dwarfness, t. When fertilisation takes place, genes T and t come together in the zygote. As T is dominant over t, all  $F_1$  progeny are tall.  $F_1$  tall plants produce two types of gametes: those carrying T and t genes. When  $F_1$  plant is self-fertilised, by combining two kinds of male and female gametes in all possible ways (as shown in Fig. 15.1), the relative proportions of offspring types are obtained. According to Mendels hypothesis, one should expect 3/4 tall and 1/4 dwarf plants in the  $F_2$  generation, i.e. in the ratio 3 tall: 1 dwarf. This is called the monohybrid ratio, since it is concerned with the inheritance of a single character. There are two types of tall plants: those carrying two T factors and those with one T factor and one t factor. The former, when fertilised, will produce only tall plants while the latter will behave like the  $F_1$  hybrid (giving 3 tall: 1 dwarf ratio).

The first test made by Mendel is known as the test cross. He crossed the  $F_1$  (hybrid (T/t)back to the dwarf (recessive) parent (t/t). The cross is diagrammed in Fig. 15.2. The diagram indicates that the expectation in the cross is 1/2 tall and 1/2 dwarf, i.e. 1 tall: 1 dwarf. Mendel actually obtained in this cross 87 tall and 79 dwarf, which is approximately 1:1 ratio as would be expected on the basis of the laws of chance. When a hybrid is crossed to one of its parents, the cross is called back cross and when the parent is recessive, it is a test cross.

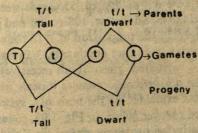


Fig. 15.2 Test cross, 1: 1 ratio.

### **Genetic Nomenclature**

### Homozygote and Heterozygote

These refer to the genetic constitution of individuals in regard to the characters inherited. An individual is termed homozygous for a given character when it has been produced by

two gametes, each bearing that character. All the gametes of a homozygote possess the character in respect of which it is homozygous. An individual is said to be heterozygous for a given character when, of the two gametes which formed it, one bears that character while the other does not. In other words, half the gametes produced by the heterozygote bear the given character. An individual may be homozygous for one or more characters and The single tienes are new esensed by determining heterozygous for others.

## Allele or Allelomorph

scale dwarf to early arrive a grow for dwarfance Allele or allelomorph is one of the series of factors or genes which are alternative in inheritance since it is at the same locus in homologous chromosomes. by combining two kinds of male and female carriers in all messages and the special to

### Genotype of a troop A Benedo vis and a private to make your entry of all the gra

It refers to the genetic make-up of an individual in regard to given genes.

### Phenotype

It is the external appearance of an individual produced as a result of interaction between genes and the environment. a local programme for the following restrict the contract of t

Law of Independent Assortment Mendel carried out a number of hybridization experiments in which two or three pairs of genes were involved simultaneously. For example, he crossed plants with round yellow seeds with those possessing wrinkled green seeds and studied the inheritance pattern of these two characters (i.e. form and colour of seed) through  $F_1$ ,  $F_2$  and  $F_3$  generations. The  $F_1$  hybrid produced round vellow seeds, indicating the dominance of these characters over their respective alleles. When  $F_1$  plants were self-pollinated, 556 seeds were obtained. Of these, 315 were round and yellow, 101 wrinkled and yellow, 108 round and green, and 32 wrinkled and green. These are approximately in the proportion of 9/16 round yellow (both dominant), 3/16 wrinkled yellow (one recessive, the other dominant), 3/16 round green (one dominant, the other recessive) and 1/16 wrinkled green (both recessive), i.e. a ratio of 9:3:3:1 (Fig. 15.3). This is known as the dihybrid ratio because it deals with the inheritance of two characters. This ratio can be best explained on the basis of a system of diagramming crosses first employed by Punnett called checker board. In Fig. 15.3, round character is represented by R and wrinkled by r. Similarly, yellow is represented by Y and green by y.

On the basis of this principle, we can compute the ratios that will be obtained in the  $F_2$ generation in respect of crosses involving more than two characters. For example, when the parents differ in three pairs of character A, B, and C, respectively dominant to a, b and c,  $F_{1}$ plants will be ABC and the F2 offspring will comprise 27 ABC, 9 ABC, 9 ABC, 9 ABC, 3 Abc, 3 aBc, 3 abC, 1 abc.

On the basis of the above results, Mendel was able to formulate his third law of independent assortment. According to this law, the members of one pair of genes segregate independently of other pairs. From this hypothesis, he deduced the results of other crosses and discovered that the data agreed with the expected ratios. He checked the hypothesis by

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making a test cross, i.e. F1 hybrid (round yellow) was crossed with a recessive parent (wrinkled green). He obtained four different kinds of offspring: 55 round yellow, 51 round green, 49 wrinkled yellow and 52 green wrinkled, which corresponds approximately to 1:1:1:1 ratio (Fig. 15.4). So it will be seen that the results obtained by Mendel are approximately according to the theoretical expectation and thus there is confirmation of the third law of independent assortment. This law makes it easy to compute the expected ratio of a cross involving any number of pairs of genes by only multiplying the data for each pair considered individually.

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|         | Round<br>yellow | Round<br>Yellow  | Round              | Round<br>Yellow    |
| 20 000  | RRYy            | RRyy             | Rryy               | Rryy               |
| Ry      | Round<br>Yellow | Round            | Round<br>Yellow    | Round              |
| ni ran  | BrYY            | RrYy             | riYY               | TITY SE            |
| rY      | Round           | Round<br>Yell ow | Wrinkled<br>Yellow | Wrinkled<br>Yellow |
|         | Rryy            | Rryy             | TETY               | rryy               |
| TY TY   | Round<br>Yellow | Round            | Wrinkled<br>Yellow | Wrinkled green     |

Fig. 15.3 Mendel's experiments on pea, 9:3:3:1 ratio. constraints and another medical and the constraints



Fig. 15.4 Test cross 1: 1:1: 1 ratio.

Besides the common pea, Mendel worked on beans, and the results obtained by him confirmed his extensive work on peas. He also carried out experiments with many other plants, and devoted considerable time to the breeding and crossing of bees but unfortunately, the record of this work has got lost. The only other published work is a brief paper

dealing with some crosses in Hieracium. His idea in this work was to obtain some of the numerous wild forms by crossing together more distant varieties and thus trace their origin and nature. But he could obtain only a few hybrids owing to the technical difficulties encountered in cross-fertilising flowers of Hieracium. Besides, the behaviour of such hybrids was quite contrary to what he had observed in peas. But at that time, it was not known that Hieracium is an apomict. as obvice and mumber of pairs of games to ours, multiplying the data to

### **Dominance**

The effects of one allele of a gene in the case of pea hybrids studied by Mendel happened to be always completely dominant to those of the alternative allele. But cases were known very early in the history of genetics where dominance was absent; for example, the history of Andalusian fowls and hybrids of red and white Mirabilis jalapa. In the latter, when a redflowered plant was crossed with a white one, the flowers of the  $F_1$  hybrids were all pink. Similarly, when a black Andalusian fowl was bred with a splashed white one, blue offspring were produced. In the case of short horn cattle, the cross between red and white coat cattle produced roan progeny. Instances were found where the hybrid offspring resembled one parent so that the dominance was incomplete. These instances point out that there may be different stages between complete dominance and the absence of dominance. These various conditions may occur among different characters of a single individual. The rule that is applicable, irrespective of the presence or absence of dominance, is that when the parents belong to true breeding varieties, the F1 hybrids are all uniform in respect of their hereditary characters. bauga!

### **Polyhybrids**

Round Wrinkled Wrinkled green reliam The offspring of hybrids may be heterozygous for many genes. Such hybrids are known as polyhybrids. In their case, the situation may be complex. As the number of genes involved in a given cross increases, the number of possible gene combinations increases fast. Every gene which is added multiplies the number of different classes of gametes produced by 2 and the number of genotypes formed in the  $F_2$  generation by 3 and the possible gamete combinations in  $F_2$  by 4.

Table 15.1 shows that as the number of genes involved increases, the change of recovering one of the original parent types in the F2 becomes rapidly less.

### Genetic Differences Among Gametophytes

It is not possible to observe any difference in the gametes in the case of higher plants or animals, with a few exceptions. These differences are inferred on the basis of the breeding data. A noteworthy exception is maize, rice and sorghum plants. Here, one can note the difference in the pollen grains, which belong to the gametophytic generation. This is the gene

Table 15.1 The relation between the number of allelic pairs in a cross and the number of phenotypic and genotypic classes in  $F_2$ 

| No. of pairs of alleles involved in the cross | No. of visibly different $F_2$ classes of individuals if dominance is complete | No. of different kinds of gametes produced by $F_1$ hybrid | No. of different genotypes | No. of possi-<br>ble $F_1$ gamete<br>combinations |
|---|--|--|----------------------------|---|
| O the baws of                                 | ottas won av tanw batt   | showed as expense  | rom dg d erp               | 16  |
|   |  | 4  | 27                         | 64  |

for waxy sperm which can also be observed in chromosome 9. Kernels homozygous for wx/wx (in the case of endosperm the genotype wx, wx, wx) have a different kind of starch than in the case of Wx/Wx and Wx/wx kernels. Kernels of the latter (Wx/Wx) and Wx/wxpossess starch which stains blue with iodine while the starch of wx/wx kernels red. This same reaction is also applicable to pollen grains. As Wx grains become blue on staining and wx grains red, it is possible to observe the gametic generation directly. Wx and wx pollen grains are formed in approximately equal numbers. This has been inferred from the breeding tests performed. They show that  $F_1$  produces 3/3 Wx—kernels and 1/4 wx/wx in  $F_2$ . Except for such cases, the study of diploid organisms is limited and it is not possible to study segregation directly in the gametophytic (n) generation. In lower plants, however, characters can be noted in the haploid generation (having one set of chromosomes), e.g. Neurospora of Ascomycetes of fungi.

DAIGABE READING

## Blending versus Particulate Inheritance

Before Mendel's laws of inheritance received recognition, biologists believed in what is called 'blending inheritance'. The mixing of germ plasms was regarded similar to the mixing of fluids, which could not again be separated into their component parts. Mendel conclusively showed that hereditary characters are carried by particulate units. These units (viz. factors or genes) are reassorted in the  $F_2$  generation, although the variability remains the same. When we evaluate the idea of blending inheritance and particulate inheritance on the basis of evolution, we find that with the former, the loss of variability in each generation is so great that there would soon be left no hereditary differences for natural selection to act on, whereas in the latter, variability is conserved and a very small mutation rate is enough to keep the population in a high variable state which is a prerequisite for occurrence of evolution.

### SUMMARY

1. Gregor Mendel is often called the Father of Modern Genetics. He was ordained as a priest at Brünn. He started his experiments on inheritance of characters in 1857. In

1866 his paper was published but it went unnoticed. In 1900, it was rediscovered simultaneously by de Vries, Correns and Tschermak.

- 2. Mendel crossed different varieties of common pea possessing sharply contrasting characters. He dealt with seven characters. He concentrated on one character at a time and studied its inheritance through several generations. Then alone, he paid attention to two or three characters together. He carried experiments with large populations of plants. From these experiments, he formulated what are now called the Laws of Mendel.
- 3. According to Mendel, hereditary characters are determined by particular units of heredity called factors (genes). The factors which are alternative in inheritance form a series and each is called an allele. The law of dominance states that the character which expresses itself is dominant and the alternative character which is not expressed is recessive. According to the law of segregation, the factors are separated during the formation of gametes and hence only one of the pairs is transmitted by a particular gamete. When two or more pairs of factors are involved, the members of one pair of factors segregate independently of other pairs. This is the law of independent assortment. Depending upon the number of characters used in hybridization experiments, Mendel obtained different ratios in the second generation (F<sub>2</sub>). The monohybrid ratio was 3:1 and the dihybrid ratio 9:3:3:1.
- 4. Besides pea, Mendel worked on beans and Hieracium. He could not succeed in the case of the latter plant.

#### **FURTHER READING**

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### 16. Interaction of Genes

Immediately after the rediscovery of Mendel's work in 1900, the laws of segregation and independent assortment were confirmed. However, in certain instances, they appeared not to hold good. This made geneticists inclined to think that these laws were not of universal application, but applied only to certain types of hybrids, such as those studied by Mendel. Transmission of characters in other crosses such as those between species or individuals differing in quantitatively varying characters were regarded by them to follow some other rules.

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29 7 Hero Complementally Center

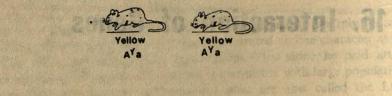
The classical Mendelian ratios such as 3:1 and 9:3:3:1 are due to complete dominance. There is independent assortment of genes and no interference between the effects of different genes. However, it is not applicable to all crosses. It was soon realised that many of the apparent exceptions could be explained on the basis of interaction of genes. Ratios were consequently modified in several ways, depending on the kind of interaction. Thus, it was shown that a broad range of characters in many kinds of plants and animals do follow Mendel's laws and the data could be analysed in terms of genes. Some of the important modifications of Mendelian mono- and dihybrid ratios are explained below on the basis of interaction of genes.

### Monohybrid Ratio 2:1

Lethal Genes in Animals

Cuenot discovered that the yellow variety of mouse never breeds true, and hence all yellow mice are hybrids. Matings between yellow mice, produce offspring which are always in the ratio of 2 yellow: 1 non-yellow. If a yellow mouse is mated with a non-yellow mouse, the progeny are always after the manner of the monohybrid test-cross, i.e. 1 yellow: 1 nonyellow. Thus showing that yellow mice are heterozygous and homozygous mice do not exist. It is because of this fact that the typical monohybrid Mendelian ratio 3: 1 is not obtained. This was first explained by Castle and Little. They found that one class of embryos from yellow by yellow matings died at an early stage. They explained that when two dominant genes interact, its effect is lethal. The homozygous yellow mice cannot survive because of the lethal effect of two doses of yellow on the embryo. The inheritance of this type is represented in Fig. 16.1.

It has been observed in Drosophila and many plants and animals that many mutant genes cause reduction in viability, with the result that the  $F_2$  population often contains a very few of the mutant types. A seytomedia and considers compared and consider an event massless.





A = Dominant yellow colour gene

a = Recessive non-yellow colour gene

Fig. 16.1 Inheritance of lethal gene in mouse.

In man, several lethal genes have been detected. One of them, juvenile amaurotic idiocy is responsible for the death of homozygous children before the age of 18.

As regards plants, let us take the example of snapdragon (Antirrhinum) to explain the action of lethal genes. Its leaves are variegated. The 'golden' variety of the plant on self-pollination forms two types of off-spring, golden and green, in the ratio 2:1. Further breeding yields green progeny which breeds true whereas the golden ones give gold and green implying their heterozygous nature. The homozygous golden does not survive because of the lethal effect of two doses of gold.

### **Dihybrid Ratios**

### 9: 7 Ratio; Complementary Genes

A very interesting type of inheritance has been observed in the case of sweet pea (Lathyrus odoratus). It possesses many true breeding varieties which were derived from the wild type of Sicily. The flowers of the wild type are purple in colour with red wings. Bateson and Punnett studied the inheritance of flower colour in many different cultivated varieties. They observed that the purple flower is dominant over white and a 3:1 ratio is obtained in  $F_2$ . The white-flowered variety breeds true. However, when two purely white varieties were crossed, they were surprised to find purple progeny in  $F_1$ , it being similar to the wild type in this respect. When  $F_1$  hybrids were self-pollinated, the ratio of 9 purple: 7 white was obtained in the  $F_2$  generation. When self-fertilised, all  $F_2$  white bred true while the purple-flowered plants after self-fertilisation gave rise to progeny of different types. Only a few bred true, some gave the ratio 3 purple: 1 white, while others formed 9 purple: 7 white as in  $F_2$ . The results are indicated in Fig. 16.2.

The above results clearly indicate that the ratio 9:3:3:1 has been modified because both the dominant genes C and P are essential for the production of purple colour. When either is absent, there is no colour formation and hence the phenotype is white. So the progeny

Common variety herry albiral

| bend all       | incus a       | Pir          |       | wolley a     | any other cost colors. And the disappearance of the                                      |
|----------------|---------------|--------------|-------|--------------|--|
| ebused we      | **********    | CCPp<br>CCPp | Cc PP | CcPp<br>Pink | are black except at the tip<br>animal is normally of a l<br>at the base. Black is recen- |
| milw<br>Cp     | ССРр          | ССрр         | Cc Pp | Ccpp         | an Thind State of Long To<br>A ratio obtained to a sale<br>A ratio obtained to a sale    |
| F <sub>2</sub> | Pink<br>CcPP  | A line       | ccPP  | white cc Pp  | egypte are distripted in the   |
|                | Pink          | Pink         | White | White        | Conjecter, they increase   |
| cp             | Cc Pp<br>Pink | Ccpp         | White | White        |  |

The inheritance of coat cohour is mouse is an example of sunglementary gener. A number

White CCPP

White

CCPP

Parents

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Fig. 16.2 Interaction of complementary gene, 9:7 ratio.

possessing both the dominant genes C and P will only have purple flowers. As per the Mendelian ratio of 9:3:3:1, the proportion of such progeny possessing genotype C/-P/with purple flowers should be 9, while other genotypes, C/-pp with proportion of 3/16, ccP/with proportion of 3/16 and ccpp with proportion of 1/16 will have only white flowers since they do not have both the dominant genes C and P. So, in this case, the terms 3:3:1 of the ratio are merged together and the result is 9 purple: 7 white.

In cases of interaction where two different genes are similar in their individual effects but are both essential for producing another and different character, the genes are known as complementary genes.

It is well known that in sweet peas and many other plants, a cross between two different white strains produces coloured progeny. Let us see how this happens. Each strain contains a gene which is a necessary component of the anthocyanin pigment. These components are different in the two different white strains. But when they come together they produce colour, by forming anthocyanian. In other words, the two genes complement each other either through crossing or a combination of components (extracts) in vitro. The outcome is the production of colour. So we assume that genes C and P are the two components involved. occurs the result is the react throng of the agouti pattern. Since the agouti mice belong to

Supplementary Genes (9:3:4 Ratio) and moistages and to the shall be stated as the same of Some cases are known where the interaction of two different genes is such that one dominant gene will produce its effect, irrespective of the presence or absence of the other gene, while the second can only produce its colour in the presence of the first. These are the cases dealing with supplementary genes.

The inheritance of coat colour in mouse is an example of supplementary genes. A number of varieties have originated from the wild grey or agouti coat-coloured mouse, the most common variety being albino, possessing a white coat colour and pink or blood-coloured eyes. This variety breeds true and its white colour character behaves as simple recessive to any other coat colour. Another variation in coat colour was produced probably through the disappearance of the yellow pigment from the agouti pattern. The hairs of this mouse are black except at the tip where they are with narrow yellow bands. The underside of the animal is normally of a lighter colour with cream or yellow hairs with some black or gray at the base. Black is recessive to agouti and breeds true. When a black mouse is mated with an albino, the offspring in the  $F_1$  generation is only agouti. When the agoutis are inbred, the  $F_2$  ratio obtained is 9 agouti : 3 black : 4 albino. The crosses are diagrammed in Fig. 16.3.

| - Sar              | Parents        |            | Caa    | cc A A<br>A Ibino |
|--------------------|----------------|------------|--------|-------------------|
| 1030               | W CA           | Ca Ca      |        | Ca                |
| CA                 | CCAA<br>Agouti | WALKERS SE | CCAA   | Cc Aa<br>Agouti   |
| Ca                 | CC Aa          | CCaa       | CcAa   | Ccaa              |
|                    | Agouti         | Black      | Agouti | Black             |
| F <sub>2</sub> c A | Cc A A         | Cc Aa      | cc A A | ccAa              |
|                    | Agouti         | Agouti     | Albino | Albino            |
| ca                 | (CcAa          | Ccaa       | cc Aa  | ccaa              |
| (5)                | Agouti         | Black      | Albino | Albino            |

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Fig. 16.3 Cross between black and albino mice, 9:3:4 ratio.

In this case, the last two terms of the  $F_2$  ratio 9:3:3:1 are added and the ratio is modified to 9:3:4. Gene C is necessary to develop any or black colour, which is absent in albino mice. Gene A is responsible for banding of the black hairs with yellow. It is absent in black mice, so it must have come from the albinos. Though albino mice contain this gene, no colour develops in the absence of gene C. But when the recombination of genes A and C occurs, the result is the reconstitution of the agouti pattern. Since the agouti mice belong to the wild type, this instance is of the reversion type.

East obtained a 9:3:4 ratio in the case of grain colour in maize. The pure red-grained plant possesses two complementary genes, C and R. The former necessary for a chromatogen while the latter for a certain enzyme. When these genes are combined, red coloured grains are produced. For the development of purple colour, another gene P is necessary.

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When gene C is absent, there is no colour formation at all. If it is assumed that gene C is constant and genes P and R are variable, the results obtained are as follows:

Purple grain 1. P and R present 2. Only R present Red grain

No colour formation 3. Only P present

### 9:6:1 Ratio (Fruit Shape in Squashes)

There are races of Cucurbita pepo (summer squash) which breed true to different fruit shapes. The spherical shape is recessive to the flat or disc shape. If two different races of spherical fruits are crossed, the  $F_1$  progeny is only of the disctype. When the  $F_1$  progeny are self-fertilised, 9/16 disc, 6/16 spherical, and 1/16 elongated types are obtained in the F<sub>2</sub> generation (Fig. 16.4). In this case, the second and third terms are added together so the dihybrid ratio is modified to 9:6:1. Here the genes affect the same character. Either A or B alone produces sphere-shaped fruits and when they are together, they interact resulting in disc-shaped fruits. In the absence of both the genes, the fruit is elongate,

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|                |       | Sp                      | 77 W 7 W 8              | pherical                |                    |
|----------------|-------|-------------------------|-------------------------|-------------------------|--------------------|
| Pa             | rents | AA                      | -shaped                 | aa BB                   |                    |
| Fi             |       |                         |                         | 100                     |                    |
|                |       | AB                      | Ab                      | ав                      | ab                 |
|                |       | Disc -<br>shaped        | Disc -<br>shaped        | Disc-<br>shaped         | Disc -<br>shaped   |
|                | AB    | AABB                    | AABb                    | AaBB                    | AaBb               |
|                | Ab    | Disc-<br>shaped<br>AABb | Spherical AAbb          | Disc-<br>shaped<br>AaBb | Spherical Aabb     |
| F <sub>2</sub> | аВ    | Disc-<br>shaped<br>AaBB | Disc-<br>shaped<br>AaBb | Spherical aaBB          | Spherical<br>aa Bb |
|                | ab/   | Disc-<br>shaped<br>AaBb | Spherical<br>Aabb       | Spherical<br>aa Bb      | Elongate           |

Fig. 16.4 Inheritance of days a summer squashes, 9:6:1 ratio.

### Other Ratios Modified by the Interaction of Different Genes

#### **Fowl Combs**

Bateson and Punnett noticed a very interesting phenomenon during their study of the inheritance of comb form in fowls. It is known that the Wyandotte breed possesses a papillate comb known as 'rose' while the Bahamas and some other varieties have a three-ridged, higher and narrower comb called 'pea'. Leghorns and other breeds with similar origin possess combs termed 'single'. Both rose and pea are dominant to single and when either of them when crossed with single, the ratio is 3:1 in  $F_2$ . However, when rose was mated with single, an entirely new form of comb appeared in the  $F_1$  and it was called 'walnut' because it resembled to half portion of a walnut meat. Such a type of comb was previously recorded as a characteristic of the Malayan variety not related either to the rose or pea varieties. Inbreeding of  $F_1$  walnuts gave 9 walnut: 3 rose: 3 pea: 1 single in the  $F_2$  generation, i.e. a dihybrid ratio. These results indicate that when two recessive genes come together, single comb is produced, while the interaction of two dominant genes results in the formation of walnut comb. When a single is mated with another single, the progeny are single, thus proving the presence of two recessive genes in the single. The crosses are diagrammed in Fig. 16.5.

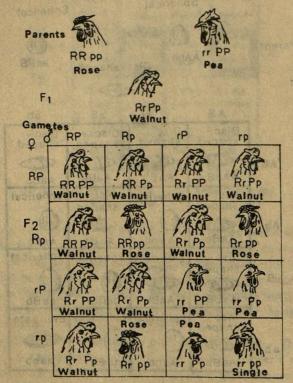


Fig. 16.5 Inheritance of comb in poultry, 9 walnut: 3 rose: 3 pea: 1 single ratio.

Figure 16.5 indicates that the interaction of dominant genes R and P is necessary for the formation of walnut comb. R alone produces a rose comb and P alone, a pea comb. When the recessive genes r and p interact, the result is a single comb. Although the  $F_2$  ratio is 9:3:3:1,  $F_1$  hybrid is dissimilar to either of the parents and two new types, walnut and single, appear in the  $F_2$  generation. R and P are two independently-inherited genes and their interaction in dominant and recessive forms produces walnut and single comb respectively.

### **Epistasis**

When two non-allelic genes affect the same part or character of an organism, the expression of one is masked by the other. The gene which inhibits the expression of another nonallelic gene is said to be epistatic to it, and the gene which is masked is said to be hypostatic. This phenomenon of the masking effect is termed epistasis. One should be able to differentiate between dominance and epistasis. Although both are concerned with masking, in the former, the two genes are allelic while in the latter they are different ones.

### 12:3:1 Ratio

The common fruit colours in summer squashes are white, yellow and green. White is dominant to both yellow and green, while yellow is dominant to green. Thus, yellow acts as a recessive to white, and as a dominant to green. If gene Y is responsible for the development of yellow colour and W is the gene which prevents its expression, the presence of the latter gene will produce white fruits. In the absence of W and Y, the colour of the fruits will be green. This type of epistasis is diagrammed in Fig. 16.6. Thus it will be seen that

| Parents        | White<br>WWYY   | WH             | reen<br>vwyy     |               |
|----------------|-----------------|----------------|------------------|---------------|
| F1 (4)         | WY              | Wy             | WY PO            | wy            |
| WY             | WW YY           | WW Yy          | Ww YY            | WwYy'         |
|                | White           | White          | White            | White         |
| F <sub>2</sub> | WW Y y<br>White | WW yy<br>White | WwYy             | Wwyy<br>White |
| wY             | Ww YY           | Ww Yy          | wwYY             | ww Yy         |
|                | White           | White          | Yellow           | Yellow        |
| endente,       | WwYy            | Ww yy          | wwyy:            | wwyy          |
| wy             | White           | White          | Yello <i>n</i> , | Green         |

Fig. 16.6 Cross between white and green summer same and the summer out the latest roughton was squashes, 12:3:1 ratio. May and squared from grider world

the ratio 12:3:1 is a modification of the 9:3:3:1 ratio, the first two terms being added together because of the presence of the dominant gene W.

#### 13:3 Ratio

The white plumage of white leghorns is almost completely dominant over the coloured plumage of black, barred, or other coloured varieties. However, the white plumage of white Wyandottes or white Plymouth Rocks is recessive to coloured plumage. It has been experimentally shown that the white leghorn is a genetically coloured bird possessing a gene for colour but unable to develop the colour because of the presence of another gene formation, i.e. it is epistatic to the colour gene. On the other hand, white Wyandottes owe their whiteness to these genes in the recessive form. Let us designate the gene for colour expression by C and the epistatic gene by I. Their interaction in this case is shown in Fig. 16.7. Since the dominant gene I does not allow the dominant gene C to have its colour

White (Leghorn) White

| Pare                 | nts   | lice           |                          | Wyando          | tte) and res si   |
|----------------------|---|----------------|--------------------------|-----------------|---|
| of July              | orden du inte<br>Organisti ( i<br>estato questi | Whi            | is an ir said            | rob a 41 bi     | nant to both yedow?<br>reservive to white, an<br>of yellow colors and |
| on and and           | IC  | IC             | The second second        |                 | letter gone will pro-<br>will be green This in                        |
| ıc                   | II CC<br>White                                  | IICc<br>White  | li CC<br>White           | liCc<br>White   |   |
| lc<br>F <sub>2</sub> | II Cc<br>White                                  | liCc<br>White  | liCc<br>White            | li c c<br>White |   |
| ic                   | liCc<br>White                                   | l iCc<br>White | iiCC<br>Colour-<br>ed    | liCc<br>Colour  |   |
| l c                  | li Cc<br>White                                  | licc<br>White  | ii C c<br>Colour-<br>ed: | iicc<br>White   |   |

Fig. 16.7 Cross between Leghorn and Wyandotte, epistasis showing 13:3 ratio.

expression, all genotypes containing I will be white, and when it is in the recessive form, gene C will be able to express itself. Therefore, in the latter condition, the birds will be with coloured plumage. Such genotypes are only three. The genotype *iicc* will develop into a white bird because the colour gene is in the recessive condition. Therefore, the

out to residence and

The state of the s

total number of white birds will be 12+1=13. In this way, the dihybrid ratio is modified The observed have desired reason rates 1 : 1 and 9 : 3 : 1 are due to to 12:3. some to them token the menching some

### Duplicate Genes (15: 1 Ratio)

another neurallette

to continue interest

ely istorised with the

There may be different genes which produce the same or almost similar effect. Such genes having the same expression are called duplicate genes. The best example of the interaction of these genes has been reported by Shull in 1914 in the plant Capsella bursa pastoris. There is a race of this species with triangular capsular fruits while another race bears ovoid or top-shaped capsules. Both the races breed true. When they are crossed, the  $F_1$ hybrid plants are with triangular fruits indicating that the triangular capsules are dominant over the top-shaped ones. Let us represent the genes responsible for the triangular shape by  $T_1$  and  $T_2$ . Since both the genes contribute to the triangular shape, the presence of even one dominant gene will make the fruit triangular. Genotype  $t_1t_1t_2t_2$ , on the other hand will develop into plants with top-shaped capsules. This will be clear from Fig. 16.8 which indicates the interaction of these duplicate genes. arried Could be decimated by articlar of Office Charle abilities and Shirts assess swift were the to

| Tr<br>Parents<br>F1                          | iangular<br>T <sub>1</sub> T <sub>1</sub> T <sub>2</sub> T <sub>2</sub>               | CANADA STATE  | t <sub>1</sub> t <sub>2</sub> t <sub>2</sub> t <sub>2</sub> t <sub>2</sub> t <sub>2</sub> t <sub>3</sub> t <sub>4</sub> t <sub>4</sub> t <sub>4</sub> t <sub>4</sub> t <sub>5</sub> t <sub>4</sub> t <sub>5</sub> t <sub>4</sub> t <sub>5</sub> t <sub>5</sub> t <sub>6</sub> t <sub>7</sub> | void<br>1 <sup>t</sup> 2 <sup>t</sup> 2<br>t1 t2                               | Actional Test  and out not 7 a  and bodeson  destines and a  test E. E. fana  and van mor 7 a |
|--|---|---|--|--|---|
| F <sub>2</sub> T <sub>1</sub> T <sub>2</sub> | T <sub>1</sub> T <sub>2</sub> T T <sub>1</sub> T <sub>2</sub> T <sub>2</sub> Triang - | T1 t2  T1T2 t2  Triang- ular  | T <sub>1</sub> t <sub>1</sub> T <sub>2</sub><br>Triang-<br>ular  | T <sub>1</sub> t <sub>1</sub> T <sub>2</sub> t <sub>2</sub><br>Triang-<br>ular | Curati cana   |
| T <sub>1</sub> * <sub>2</sub> ,              | T <sub>1</sub> T <sub>1</sub> T <sub>2</sub> t <sub>2</sub><br>Triang-<br>ular        | T <sub>1</sub> T <sub>1</sub> t <sub>2</sub> t <sub>2</sub><br>Triang -<br>ular | T <sub>1</sub> t <sub>1</sub> T <sub>2</sub> t <sub>2</sub><br>Triang -<br>ular  | T <sub>1</sub> t <sub>1</sub> t <sub>2</sub> t <sub>2</sub><br>Triang-<br>ular | mate Percentage 1 14  |
| t <sub>1</sub> T <sub>2</sub>                | T <sub>1</sub> t <sub>1</sub> T <sub>2</sub> T <sub>2</sub><br>Triang -<br>ular       | T <sub>1</sub> t <sub>1</sub> T <sub>2</sub> t <sub>2</sub><br>Triang-<br>ular  | thtT2T2<br>Triang .<br>ular  | titiT212<br>Triang<br>ular   | the F. procession<br>indicated and war<br>F.2. In rich, when the s<br>200 years with          |
| tite   | T <sub>1</sub> t <sub>1</sub> T <sub>2</sub> t <sub>2</sub><br>Triang _<br>ular       | T1111212<br>Triang  | t <sub>1</sub> t <sub>1</sub> T <sub>2</sub> t <sub>2</sub><br>Triang-<br>ular   | t <sub>1</sub> t <sub>1</sub> t <sub>2</sub> t <sub>2</sub><br>Ovoid           | 4 she fir court clear  F. 7 to control wellow a  con What will b                              |
|  |   | T MALEULINE   |  | Cansella   | P. s. In cotton, the inher  |

Fig. 16.8 Inheritance of capsule shape in Capsella bursa pastoris indicating interaction of duplicate genes; 15 : 1 ratio.

Maria Contractor In fact, the ratio is  $9T_1/-T_2: 3T_1/-t_2t_2: 3t_1t_1T_2/: 1t_1t_1t_2t_2$ , but since in all the genotypes except  $t_1t_1t_2t_2$  there is a dominant gene  $T_1$  or  $T_2$ , or both, the phenotype is triangular. So the ratio is modified to 15: 1 by merging the first three items (9+3+3). (c) xx K, K, K, K, K,

#### SUMMARY

- 1. The classical Mendelian ratios like 3:1 and 9:3:3:1 are due to complete dominance. There is independent assortment of genes and no influence between the effects of different genes. However, after 1900, instances were found where such ratios were not obtained. It was, however, realised that these cases could be explained on the basis of interaction of genes. Ratios were modified in several ways depending upon the kind of interaction of genes.
- 2. The monohybrid ratio of 2:1 observed in the case of mouse is due to the action of lethal genes. The ratio 9:7 obtained in sweet pea is due to the interaction of complementary genes. In the case of 9:3:4 ratio, the interaction of supplementary genes is responsible. The inheritance of coat colour in mouse is an illustration of this ratio. It also explains reversion.
- 3. In the case of 9:6:1 ratio in summer squash, the genes affect the same character and produce sphere-shaped fruits when they come together. The result is disc-shaped fruits. In poultry, the inheritance of comb pattern is interesting. In this case, when two recessive genes come together, single comb is produced. Interaction of two dominent genes results in the formation of walnut comb.
- 4. When two non-allelic genes affect the same part of the character, the expression is masked by the other. The gene which inhibits the expression of another non-allelic gene is epistatic to it and the phenomenon is called epistasis. The instances of 12:3:1 and 13:3 ratios are examples of epistasis.
- 5. There may be different genes producing the same or almost similar effect. Such genes are called duplicate genes. Inheritance of triangular and ovoid capsular fruits in Capsella bursa pastoris is the best example of the interaction of duplicate genes.

### PROBLEMS

- P.1 In castor-oil plant, when a spineless capsule mutant was crossed with a normal spiny capsule plant the F<sub>2</sub> generation had 168 plants with spiny capsules and 52 without them. How was this character, inherited, and what was the ratio?
- P.2 In rice, when the short glume variety was crossed with the long glume variety, the F<sub>2</sub> generation had 200 plants with short glumes and 16 with long glumes. (i) Find out the genotypes of the parents. (ii) What will be the result if the F2 plants are crossed with long glume plants? (iii) What will happen if the F2 short-glume plants are self-fertilised?
- P.3 In maize, yellow endosperm is dominant over white endosperm and starchy endosperm over the sugary one. What will be the phenotype of F<sub>1</sub> if the yellow, starchy endosperm variety is crossed with the white, sugary endosperm variety? What will be types in F2?
- P.4 In cotton, the inheritance of colour is very complex. There is a basic dominant gene responsible for the colour of the cotton and there are different genes for producing a specific colour. Brown colour is produced by the interaction of genes  $K_1$  and  $K_2$ . If only one of them is present, the cotton will be cream-coloured.

Find out the phenotypes of the parents, the  $F_1$  genotype, the  $F_2$  phenotypes, and their ratios in the following crosses.

(a)  $xx K_1K_1k_2k_2$ xx k1k1K2K2

(b) xx K1K1k2k2 xx k1k1ksks (c)  $xx K_1K_1K_2K_2$ 

 $xx k_1k_1k_2k_2$ 

- P.5 In black gram, the cross between colourless hair and coloured hair varieties yielded 65 plants with brown hair and 79 with colourless hair in the  $F_2$ . Determine the genotypes of the parents, and the  $F_1$ and  $F_2$  off spring.
- P.6 In rice, production of anthocyanin in the leaf margin is controlled by two genes. A cross between green margin and pink margin results in the F<sub>1</sub> with pink margins and in the F<sub>2</sub> 95 plants with pink and 73 with green leaf margins. Determine the genotype of the parents.
- P.7 In mung, when a black seed variety was crossed with a yellow seed variety, the F<sub>1</sub> was with brown seeds. When F<sub>1</sub> was self-fertilised, 200 plants with black seeds, 74 with brown seeds and 85 with yellow seeds were obtained. Comment on the inheritance of seed colour.
- P.8 In maize, white seedling is lethal and recessive. For lethality, several independent genes are responsible. While research was carried on in this connection, it was observed that when two plants are crossed, 122 green and 90 white seedlings are obtained. (i) Give the genotype of the parents. (ii) If 100 plants in the above with green seedlings are self-fertilised, what kind of plants, and in what proportion, will be obtained? (iii) In another experiment, in the segregating offspring the ratio was 15 green: 1 white seedling. Explain this result.
- P.9 In poultry, the gene for rose comb is R and for pea comb P. When these genes come together, a walnut comb is formed. But when the recessive alleles of these genes are present, the result is a single comb. Determine the kinds of comb and their proportions in the following crosses.

| RrPp×RRpp | rrPP×RRpp |
|-----------|-----------|
| RRPp×rrPP | Rrpp×rrPP |
| RrPP×RRPp | RrPp×rrPp |

- P.10 The crosses in respect of comb pattern in poultry yielded the following results.
  - (i) In a cross of walnut with pea, 3/4 walnut and 1/4 rose were obtained.
  - (ii) In a cross of walnut with rose, 1/2 walnut and 1/2 pea were obtained.
  - (iii) In a cross of rose and walnut, 1/2 walnut, 1/4 rose and 1/4 single were obtained.

Determine the genotypes of the parents.

P.11 In sweet pea, if genes C and P come together, coloured flowers are produced. If either of them is absent, the flowers are white. Determine in the following crosses the type of flowers and their proportions in the offspring.

ccPP×CCpp CCPp× ccpp CcPP×CCPp CcPp×ccPp

- P.12 In sweet pea crossing experiments, the following progeny was obtained:
  - (a) When a white-flowered plant was crossed with another white-flowered plant, the progeny was in the proportion of 1/2 coloured flowers and 1/2 white flowers.
  - (b) In a cross of white-flowered plant with coloured-flowered plant, the offspring obtained were in the proportion of 3/4 coloured and 1/4 white flowered plants.

Determine the genotypes of their parents.

P.13 In mice, when the gene C for black skin and the gene A for white skin (albino) come together, the result is an agouti mouse. Find out the phenotypes and their proportions in the following crosses.

Ccaa× ccAa CcAa× CCaa CCAa×ccAA CcAA×CcAa

- P.14 In rice, when plant A is crossed with plant B, the offspring were 120 red grain: 40 grey grain. But when A was self-fertilised, the offspring were 180 red grain: 120 grey grain. Determine the genotypes
- P.15 In jowar (Sorghum vulgare), genes P and Q interact to produce glume colour. Blackish pink colour is due to Pp. When Q and P come together, the glumes are reddish pink. The own effect of Q is not seen if P is absent. Hence, pQ and pq glumes are brown. Determine glume colour in the offspring and their proportions in the following crosses.

Ppqq × ppqq PpOq× PpQq ppQQ × Ppqq PPOq× PpQq

P.16 In jowar, red grain is dominant over pearl grain. Red colour is due to the gene W and pearl grain is due to the gene Z. In the presence of W, Z is ineffective. But when recessive genes w and z come together, the grain is like white chalk. Determine the grain colour and proportions in the following crosses.

WwZz×WwZz
WwZz×wwZz
WWzz×wwZz
Wwzz×wwZz
Wwzz×wwZz

P.17 In the crosses mentioned in Problem 16, the following results were obtained.

(i) When the red grained plant was crossed with a white chalk-coloured plant, 1/2 red grain, 1/4 white pearl and 1/4 white chalk-coloured grain progeny were obtained.

(ii) A cross between red grained and pearl grained plants, produced only red grained offspring.

Determine the genotypes of the parents.

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## 17. Mendel and the Chromosome Theory of Heredity

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The relation between Mendel's work and chromosomes is well known. But during Mendel's time, cytology was in its infantile stage. Nothing was known about chromosomes, mitosis and meiosis. It is not surprising therefore that Mendel did not know that genes are situated in chromosomes. It was much later that Benedin, Flemming, Strasburger, etc. discovered that homologous chromosomes pair in meiosis and segregate during gamete formation. On the basis of his experiments with pea Mendel proposed the hypothesis that the contrasting characters like tallness and dwarfness in pea are determined by certain units. They are transferred from parents to the offspring through gametes or sexual cells. He referred to these units as factors (now, genes). Mendel was the first to show how genes are distributed in heredity. When chromosomes were discovered and their behaviour during meiosis known, compared with the behaviour of Mendelian factors, it was found to be the same. Biologists inferred from this that the factor might be situated in the chromosome.

## The Chromosome Theory

This theory was first proposed by Bovery and Sutton in 1904, and later supported by Wilson. Subsequently, Morgan and his students elaborated it.

When Sutton first put forward his arguments in this connection in 1902, they were so cogent that biologists were convinced that chromosomes are the carriers of Mendelian factors. His arguments are summarised below.

- 1. As the spermatozoon and the egg cell form a bridge from one generation to another, they must be bearers of hereditary characters.
- 2. When the spermatozoon matures, it is devoid of almost all cytoplasm. As the contribution of the spermatozoon or sperm is equal to the contribution of the egg in heredity, it is evident that the hereditary factors must be present in the nucleus.
- 3. At the time of mitosis, the only visible parts in the nucleus which accurately divide are the chromosomes. Hence, it can be concluded that genes are situated in the chromo-
- 4. Chromosomes are in pairs, so also are Mendelian factors.
- 5. During meiosis, the chromosomes are segregated, i.e. each homologue in the pair separates and the members of the pairs go to different cells. Mendelian factors also separate at the time of gamete formation. the surpresentation of the first too is the present entering and the configuration of the con

teast in some of their tiers should be more than one uses. We assume that I and to spher are present in the love maternal chromosome and who the short chromosome. The major the straining of the secondary is come to the idea is appointed by an intercent of present

6. The members of one homologous pair of chromosomes segregate independently of the members of the other pairs. Similarly, Mendelian factors responsible for one pair of contrasting characters assort independently of the factors of other pairs.

In other words, the behaviour of chromosomes is as per Mendel's laws. On the basis of the above arguments, Sutton showed convincingly how cytology and genetics are closely related. Since then cytogenetics has come into being.

The principles relating to gene segregation and independent assortment explained by Mendel are applicable to the inherit acc of several characters. This was verified when Mendel's work was rediscovered. However, in 1906 Bateson and Punnett found an exceptional example. While experimenting on sweet pea, they observed that two pairs of alleles did not assort independently. If two alleles A and B are from the same parent  $(AAbb \times aaBB)$ , their tendency will be to enter into different gametes. In the case of diheterozygotes, two different terms are used. If the arrangement is AB/ab, it is called coupling and if it is Ab/aB, it is termed repulsion. In the former arrangement, both the dominant genes are in the same chromosome, while in the latter arrangement, one dominant and one recessive gene are in the same chromosome. No satisfactory answer could be given for this till Morgan explained the phenomenon in 1910. While working on Drosophila, he noticed a similar type of event. From this he drew the conclusion that coupling is the same as linkage. However, we shall deal with this in the next chapter.

When Morgan and his associates began research in genetics, they used the common fruitfly (Drosophila melanogaster). It proved to be as useful to them in their work as the pea was to Mendel. After Morgan, many geneticists successfully used the fruitfly in their researches.

According to the chromosome theory, chromosomes are the physical basis of heredity. Genes, which are responsible for the expression of characters, are situated in the chromosomes. From there, they influence the cells and the organism constituted by the cells. They are arranged in a linear fashion in the chromosome, like beads in a necklace. The genes have a specific place in the chromosome. It is called the *locus*. This term is used for indicating the location of a gene on a chromosome as well as for designating the gene whose variants act as alleles. In the latter sense, the locus is almost identical to gene. This is the broad outline of the chromosome theory.

Let us see how we can explain the process of segregation on the basis of the chromosome theory. Gametes are produced in the process of meiosis. Chromosomes in a gamete may differ from one other in their size and form. In such a case, the gamete may be female or male, and it possesses the corresponding series of chromosomes. Because of this, the zygote contains the series of chromosome pairs. In each pair, one member is from the corresponding pair in the female gamete and the other member from the corresponding pair in the male gamete.

Let us consider that A and B are two Mendelian factors in the original female gamete, while in the male gamete of the parent there is none. After the fertilisation of these gametes, the genotype of the zygote will be AaBb. It will form four types of gametes, AB, Ab, aB, and ab. If we assume that every different gene is in a different chromosome pair, the assumption will be according to the chromosome theory, and if not in all the chromosomes, at least in some of them there should be more than one gene. We assume that A and C genes are present in the long maternal chromosome and B in the short chromosome. The individuality of the chromosomes is conserved. This idea is supported by an independent proof

If this is so, than A and C genes should always be linked together. If they separate from each other, then we have to either reject the chromosome theory or provide some other explanation. Morgan put forth such an explanation in the form of an hypothesis.

In Drosophila, there are more than 400 different genes, while there are only four chromosomes in a gamete. Morgan and his associates performed breeding experiments on millions of Drosophila flies. On the basis of these experiments, they concluded that all the genes present in the fly can be divided into four groups. All the genes in the same group indicate linkage with each other but not with genes present in the other group. In other words, the four groups of genes correspond with four chromosome pairs. This can be proved on the basis of sex linkage. Of the four chromosomes in a gamete, one is very short. The three groups of genes are accommodated in chromosomes 1 to 3, and each chromosome contains many genes. This means that larger the number of genes, longer the chromosome. This is exactly the condition of these chromosomes. While in the case of chromosome 4, which is very small, there are only a few genes. The genes situated in this chromosome show sex linkage inheritance whereas the other three chromosomes do not. This fact indicates that genes of this group are situated in the sex chromosome. We shall consider this aspect in detail in the chapter on sex linkage. affected annual former, about a remine grander method because and a membrane some remainder and a contraction of

## THE SUMMARY MANAGEMENT SUMMARY

- 1. There is a relation between Mendel's work and chromosomes. When chromosomes were discovered and their behaviour during meiosis was known, it became clear that their behaviour was similar to that of Mendelian factors.
  - 2. The chromosomes theory was first proposed by Bovery and Sutton in 1904. Sutton's arguments in this connection were so convincing that it was accepted that chromosomes are carriers of Mendelian factors. Their behaviour is as per Mendel's laws.
  - 3. In 1906, Bateson, and Punnett found an exceptional example in sweet pea. They observed that two pairs of alleles did not assort independently. Here, they found what was called coupling and repulsion. If the arrangement is AB/ab, it is coupling and if Ab/aB, it is repulsion. Morgan who explained this phenomenon in case of Drosophila stated that coupling and repulsion are two aspects of the same process.
  - 4. The work of Morgan and his associates on Drosophila melanogaster resulted in the formation of the chromosome theory. According to it, chromosomes are the physical basis of heredity. Genes are arranged linearly in the chromosome. They have a specific place in the chromosome, which is called locus. The process of segregation is explained on the basis of chromosome theory.

### FURTHER READING

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Wilson, E.B. (1937), The Cell in Development and Heredity, Macmillan and Co., London.

# 18. Linkage and Crossing Over

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The crosses that we have studied so far indicate that different pairs of genes are inherited independently of each other. However, it is not true for all cases.

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Mendel was fortunate to have chosen the garden pea as the experimental material as it has seven pairs of chromosomes, although he was unaware of the fact that the seven pairs of characters which he had selected happened to have their genes located in different chromosomes. Since the genes were situated in different chromosomes, they could assort independently of each other.

Had Mendel noticed an association of two characters, he might not have perhaps even understood it. Bateson discovered what was called 'gametic coupling' between two dominant genes in the sweet pea. He observed the gametic ratio of 7 BL: 1 Bl: 1 bl: 7 bL as regards blue colour and long pollen grain characters. He was, however, unable to identify the parental type producing the odd gametic ratio since he crossed two white varieties, which yielded a purple progeny. He never thought of associating gametic coupling with physical location of genes within a chromosome. Had Mendel studied the inheritance of one more character, he might have noted an exception to his law of independent assortment.

### Linkage

It was Bateson and Punnett (1905) who first discovered an exception to the law of independent assortment. They observed two pairs of alleles in sweet pea not assorting independently. They explained this behaviour on the basis of coupling and repulsion (refer to Ch. 17 for an explanation of these two terms).

We also noted that a satisfactory explanation was given by Morgan on the basis of linkage. He discovered that when two genes are located in the same chromosome, they tend to stay together in inheritance. This process is known as linkage. Morgan assumed that this tendency of linked genes of staying in their original combination was owing to their residing in the same chromosome. He also put forth the idea that the degree or strength of linkage depends on the distance between the linked genes in the chromosome. This idea led to the chromosome theory, which became the basis for the construction of genetical or linkage maps of chromosomes.

### Linkage in Drosophila

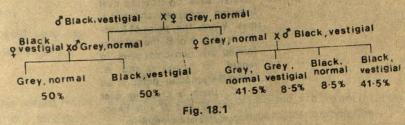
Grey body colour as against black body colour and normal wings as opposed to vestigia wings are the characters found in *Drosophila*. In the case of the vestigial form, the wings

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are so much reduced in size that the insect is unable to fly. Grey body and normal wings are dominant over black body and vestigial wings respectively. The genes for these characters are located in one of the two long pairs of chromosomes, i.e. chromosome II.

When a black fly with vestigial wings is crossed with a grey normal one, the  $F_1$  progeny is grey normal. The reciprocal cross also gives the same result. If in a test cross, an F<sub>1</sub> male is mated with a double recessive, grey normals and vestigials are obtained in equal numbers as per expectation. This is because the  $F_1$  male contained one chromosome derived from the grey normal parent and the other chromosome from the black vestigial parent. When these homologous chromosomes belonging to the second pair separate in meiosis to form gametes, half the number of gametes will contain a grey normal chromosome and the other half will have a black vestigial chromosome. So when these gametes unite with the black vestigial gametes produced by the double recessive parent, the expected ratio 1 grey normal: 1 black vestigial results. The genes for grey body and normal wing remain together, indicating thereby that the integrity of the chromosome in which they reside is preserved.

However, when a  $F_2$  female is crossed with a double recessive black vestigial male, four types of offspring are obtained, namely, grey normal, grey vestigial, black normal and black vestigial. Moreover, two kinds-grey normal and black vestigial-are about five times more than the other two kinds of offspring. These results are diagrammed in Fig. 18.1.



The resulting ratio in the case of a cross between a grey normal female and a black vestigial male is greatly different from the typical test cross ratio 1:1:1:1.

The preponderence of parental types grey normal and black vestigial can be explained on the basis of coupling, since the tendency of the genes situated in the same chromosome is to stay together in inheritance. But how is one to explain the appearance of grey vestigial and black normal types? The genes for grey body and normal wings are located in the same chromosome while those for the black body and vestigial wings are located in the other chromosome of the pair. But how is that the genes in the same chromosome are dissociated from one another? Why is the behaviour of an  $F_1$  female different from that of an  $F_1$  male?

### **Crossing Over**

Morgan and his associates answered the above question by formulating an ingenious hypohesis according to which there is interchange of material between the paternal and maternal nembers of the pair of chromosomes. This process involves some kind of exchange of egments between the homologous chromosomes. It is called crossing over. For interpreting nkage of genes, Morgan and his associates have assumed that during meiosis when homolo-

gous chromosomes synapse, there is transference of material from one chromosome to the other. This will be clear from Fig. 18.2.

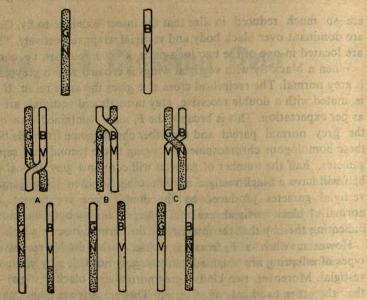


Fig. 18.2 Diagram showing crossing over between synapsed chromosomes. When the point of junction is as in A or B, the gametes formed are non-crossovers in regard to genes G and N.

When the junction point is as in C, there is crossing over between G and N and hence the gametes are crossovers.

You will now understand that the offspring 'grey, vestigial' and 'black, normal' can be produced only through the process of crossing over of segments between the chromosomes in the heterozygous parent. These offspring are the result of the new combinations of linked genes, and are called *recombinants* and the process *recombination*.

In crosses between grey normals and black vestigial females no recombinants were obtained because no crossing over occurs in the male *Drosophila*.

There exist, in an organism, many more different genes than there are pairs of chromosomes. Hence, at least some chromosomes must possess many genes. All these genes do not segregate independently. In other words, the linked genes lie in the same chromosome.

#### Method of Notation for Linked Genes

Hutchinson studied linkage in maize. He selected one variety which had coloured and normally filled grains (full) while the other selected variety was with coloured and shrunken grains. He had already observed while doing some other experiments that the colour gene C

was dominant over the colourless gene c and the full grain gene S (full shape of endosperm) was dominant over shrunken s. So the genotypes of the two varieties were CCSS and ccss. He crossed these two varieties and obtained an  $F_1$  hybrid with coloured and full grains. Its genotype was CcSs. After test crossing, the following progeny was produced.

| ons than   | Phenotype            | No. of seeds   | Genotype                              |
|------------|----------------------|--|---------------------------------------|
| ni degal   | Coloured, full       | 4032   | CcSs                                  |
| 2.         | Coloured, shrunken   | 149  | Ccss                                  |
| recold 3.1 | Colourless, full     | 152  | ccSs                                  |
| 4.         | Colourless, shrunken | 4035   | once cess                             |
|            | То                   | tal 8368   | 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 |
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Recombinants:

The above results indicate that the two pairs of genes Cc and Ss have not assorted independently but their tendency is to be inherited in their own parental combination.

The final proof for the linked properties of genes was obtained when a reverse cross was made in maize. This cross involved two varieties: (i) 'colourless, full'; and (ii) 'coloured, shrunken' seeds. Let us now diagram the process.

Colourless, full Coloured, shrunken

$$\frac{cS}{cS} \times \frac{Cs}{Cs}$$

Eggs  $cS$  Male gametes  $Cs$ 

Coloured, full

 $\frac{cS}{Cs} - \frac{cS}{Cs}$ 

Coloured, full Colourless, shrunken

 $\frac{cS}{Cs} - \frac{cS}{cs}$ 

Test cross  $\frac{cS}{Cs} - \frac{cS}{cs}$ 

#### Progeny

| Phenotype            | No. of seeds | Genotype | Percentage |
|----------------------|--------------|----------|------------|
| Coloured, full       | 639          | CcSs     | 1.47       |
| Coloured, shrunken   | 21379        | Ccss     | 48.53      |
| Colourless, full     | 21909        | ccSs     | 48.53      |
| Colourless, shrunken | 672          | ccss     | 1.47       |

The above results indicate that the parental combinations are far more numerous than the recombinants. Of course, these parental combinations are just the opposite of those in the first experiments.

Note the manner of representing linked genes. For example, the genotype of 'colourless, full' in the parental generation is written as  $\frac{cS}{cS}$ . This is a simplification of the representa-

tion  $\frac{cS}{cS}$  where the double lines indicate the homologous chromosomes in which genes c and

S are residing. The designation of  $F_1$  individuals as  $\frac{cS}{C_S}$  means that genes c and S are is one homologue and C and s are in the other.

If one examines the results of these crosses carefully, he/she will find that the 'coloured, shrunken' and 'colourless, full' in the test-cross progeny are determined by egg cells possessing the same allele-in-chromosome combinations as had occurred in the different gametes of the parental generation and also in the chromosomes of the  $F_1$  females acting as parents in the test cross. Egg cells which have given rise to 'colourless shrunken' or 'coloured full' in the test-cross offspring show a recombination of the original parental arrangement of alleles.

For the estimation of linkage values, test-cross data should be used instead of  $F_2$  data since from the former the totals of recombination and non-recombination types of gametes can be read directly.

# Chromosome Theory and Crossing Over

The essential principle of the chromosome theory has already been dealt with earlier. Let us now examine it with reference to the linkage and crossing over. In this connection, the points which have been discussed lead to the following conclusions.

- 1. Genes are situated in chromosome like beads in a necklace.
- 2. When a gene A and its allele a occur in different members of a pair of homologous chromosomes, the gene and its allele occupy corresponding places in the homologues.
- 3. For producing recombination between two different allelic pairs residing in the same chromosome pair, it is necessary to have crossing over between the locations (loci) of the genes involved.
- 4. We are mostly concerned with the crossing over that takes place in meisosis—I. However, the occurrence of somatic crossing over has also been recorded, but this is an exception.

5. Crossing over occurs during meiosis at the four-strand stage (pachytene to diplotene), i.e. when four chromatids are present for each pair of the chromosomes.

Figure 18.3 explains the occurrence of crossing over on the basis of linkage.

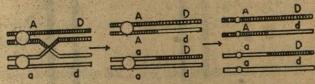


Fig. 18.3 Crossing over (diagrammatic) between homologous chromosomes and exchange of corresponding segments in regard to genes A and D. L-R-Crossingover taking place at pachytene stage of meiosis. The stage after completion of meiosis-i. Tetrads produced after meiosis-II. First and fourth chromatids are non-crossovers whereas second and third are crossovers.

# Crossing Over at the Four-Strand Stage

If crossing over takes place during meiosis, when the homologous chromosomes are in close association, there would be two possibilities—crossing over might occur either at the twostrand stage or when they had doubled, i.e. at the four-strand stage. Enough evidence is available to indicate that crossing over takes place at the four-strand stage. In the haploid fungus Neurospora of the Ascomycetes, the phenotypes can be directly determined by examining the ascocarps, which are the direct products of meiosis. In Drosophila also, occurrence of crossing over at the four-strand stage has been demonstrated, but indirectly.

# Cytological Proof of Crossing Over

In 1931, Kurt Stern gave cytological proof of crossing over. He used aberrant X-chromosomes in Drosophila. There was one X-chromosome to which a portion of a Y-chromosome was attached, while the other X-chromosome was a broken one. Its accentric portion was attached to the chromosome A (autosome). Both kinds of aberrant chromosomes could be easily identified and differentiated from the normal under a microscope. Stern crossed the female Drosophila carrying these abnormal chromosomes and heterozygous for carnation (a recessive eye-colour variant) and for bar-eye (dominant) with carnation males. When he examined the chromosomes of the progeny, he found that the genic recombination was accompanied by appropriate exchange of chromosome segments which could be easily identified. A look at Fig. 18.4 will make this clear.

Sometimes, crossing over occurs at two points in the same chromosome (Fig. 18.5). This is called double crossing over. The gametes produced as a result of double crossing over are known as double cross overs. The usual type of crossing over, i.e. crossing over just once, is

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called single crossing over and the gametes formed are termed single cross overs. With increasing distance between two loci, the amount of double crossing over also increases. However, as a rule, double cross overs are fewer than single cross overs.

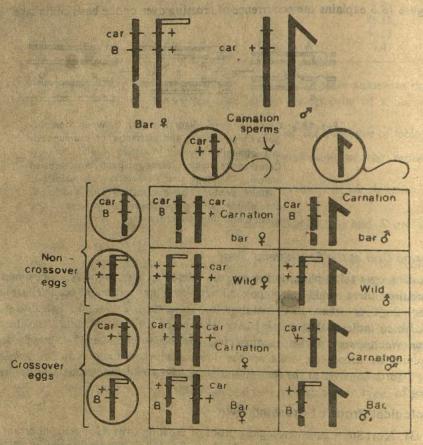


Fig. 18.4 Cytological proof of crossing over. X-chromosome aberration observed by Kurt Stern (after Stern, 1931.)

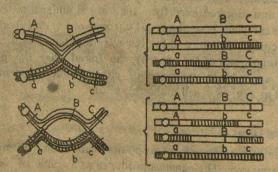


Fig. 18.5 Double crossing over.

Crossing over might take place also at three points in the same chromosome pair. This is called triple crossing over. Over short distances, triple cross overs are very few, if any. Ouadruple cross overs are even less than triple ones.

## Chromosome Mapping

Crossing over takes place at various points along the chromosome more or less randomly. Therefore, if the two genes are very close to each other, crossing over will be very rare. On the other hand, it they are wide apart, it will be more frequent. If we assume that the incidence of crossing over between two genes a and b is twice as frequent as between c and d, it is reasonable for us to infer that the distance between a and b is twice that between c and d. In other words, it is possible to arrange genes in a chromosome on the basis of their cross over distance.

Assume there is 15% recombination between genes A and B, and 10% between B and C. If these genes are to be located in a chromosome, there is only one arrangement which is compatible with the above figures—that shown below:

If another gene D has 12% recombination between C and D and 22% between B and D, the arrangement of these four genes will be as follows:

In this way, one can construct a gene map of an entire chromosome. The map distances are measured in units of 1% crossing over. If there is 10% crossing over between two genes, it will mean that they are 10 units apart and an average of 0.10 cross overs between them. The distances between genes based on cross over percentages are approximate since crossing over does not occur with equal frequency in all parts of the chromosome. The order of genes is, however, correct. These maps are known as chromosome maps.

Between 1910 and 1916 Morgan and his co-workers developed the idea that genes are arranged linearly in the chromosome and their distance apart could be measured in terms of cross-over values. Since then, numerous experiments have been made on different plants and animals and they have proved that the idea is correct.

#### Three-Point Test Cross

Suppose that two genes D and E are situated far apart in the chromosome and two exchanges have taken place between a pair of chromatids, as indicated in Fig. 18.6. You will observe that the recombination effect of the marker genes is not shown by the double cross-over chromatids. Actually, these cross-over chromatids are indistinguishable from non-cross overs. As the map distance concept is based on the number of actual physical exchanges, recombination values may give a very low estimate of the distance between genes if there is frequent occurrence of double crossing over. In order to avoid this possibility, geneticists employ a method called the three-point test cross which gives reliable recombination data. In this method, three different genes located within a relatively short segment of a chromosome are considered. One of the advantages of this method will be clear from Fig. 18.6. If a third gene pair Ff between D and E is segregating, we cannot get a true picture from the upper figure but the lower figure, involving three different genes indicates that double crossing over between D and E can be detected by the alteration of relationship of the middle allelic pair Ff.

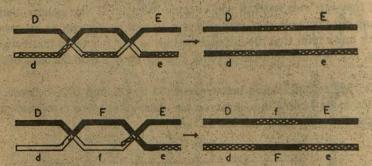


Fig. 18.6 Double crossing over between two allelic pairs, *D* and *E*. Since in the upper diagrams, only two allelic pairs in the same chromosome are available as markers, a double cross over between two chromatids is not genetically detected (right hand diagram). In the lower diagrams, there is a third allelic pair, *F* situated in the segment exchanged and so the double cross-over is detected.

In order to understand how the three-point test cross method is utilised for mapping genes, let us consider the following example:

In the test-cross

the following offspring were obtained:

$$+++|abc| = 360$$
  $++c|abc| = 94$   
 $+bc|abc| = 45$   $+b+|abc| = 6$   
 $a++|abc| = 55$   $ab+|abc| = 96$   
 $abc|abc| = 340$   $a+c|abc| = 4$ 

It is the practice to denote dominant genes by the sign '+'. If a map of these genes is to be constructed, the calculations will be done as follows.

The female parent will form 8 types of gametes after meiosis. Of these two gametes + + + and abc will be non-cross overs and the remaining 6 will be cross overs. Two will represent

single cross overs namely, +bc and a++ in the region I and the other two, ++c and ab+in the region II. The double cross overs will be +b+ and a+c.

The male parent being recessive will produce only one type of gametes having the genotype abc.

The non-cross over progeny due to linkage will be the largest in number and the double cross overs the least.

The total progeny due to single cross over in region I = 45 + 55 = 100. The percentage of single cross overs in this region =  $\frac{100}{1000} \times 100 = 10$ .

The total frequency due to single crossing over in region II = 94 + 96 = 190.

The percentage of single cross overs in region II =  $\frac{190}{1000} \times 100 = 19$ .

The total frequency due to double crossing over = 6 + 4 = 10. The percentage of double cross overs =  $\frac{10 \times 100}{1000} = 1$ .

It is necessary to add the percentage of double cross overs to each of the percentages of single cross overs.

Total % of cross overs in region I = 10 + 1 = 11

Total % of cross overs in region II = 19 + 1 = 20.

Since the crossing over in region I is between a and b and its percentage is 11, the distance between them will be 11 map units. Similarly, crossing over in region II being 20%, the distance between b and c will be 20 map units. Therefore, the map for these genes is

## Interference

In the above example, the cross-over frequency in the region I is 0.11, and 0.2 in region II. If these represent the probabilities of cross overs in these two regions and if these are independent events, the probability of double cross over is  $(0.2) \times (0.11) = 0.22$ . However, the proportion of double cross overs actually observed is 0.01. This is less than the expected amount. Usually, in the experimental data, the proportion of double cross overs is less than would be expected on a random basis. This difference indicates that the occurrence of one cross over apparently interferes with the occurrence of other cross overs in adjacent regions. This phenomenon is called interference. The observed number of double cross overs divided by the expected number is known as the coefficient of coincidence. It is a measure of indicating the amount of interference.

# Determination of Gene Order in a Three-Gene Cross

If the order of the genes in a chromosome is unknown, it can be easily determined. Let us consider the test cross between the triple heterozygote (ABC)/(abc) and the recessive homozygote (abc)/(abc), where the order of the genes in the bracket is unknown. The total test cross progeny was 1000 and their types were as follows:

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| ABC | 310 | Abc   | 120  |
|-----|-----|-------|------|
| abc | 290 | aBc   | 130  |
| ABc | 4   | AbC   | 75   |
| abC | 6   | aBc   | 65   |
|     |     | Total | 1000 |

For simplification, only the chromosomes from the heterozygous parent are given above because the contribution from the homozygous parent is always abc.

One method of determining the gene order is from the double cross over class. In the above mentioned data, 4 and 6 are the smallest numbers. Therefore, ABc and abC are the double cross overs. Note that if C and c are interchanged, you will get non-cross over gametes. Therefore, C must be in the centre and the gene order is ACB or BCA. Once the gene order is determined, the usual procedure already given for calculating distance is to be followed.

#### **Absence of Crossing Over**

Crossing over has been reported in both sexes of most plants and animals studied, including man. There are, however, a few exceptions. For example, in male *Drosophila*, crossing over does not take place. We do not know the reason for its non-occurrence. In the female silk moth also, there is no crossing over. You should note that the female is XY in moths.

#### Chromosome Maps and Linkage Groups

A gene in a given chromosome indicates the linkage relations with other genes located in the same chromosome. It is, however, transmitted independently of genes situated in other chromosomes. So each gene is the member of some one linkage group consisting of a number of genes held together by the physical continuity of the chromosome. It, therefore, follows that the number of linkage groups corresponds to the haploid number of chromosomes, i.e. different kinds of chromosomes characteristic of the species. The haploid number is 4 in Drosophila melanogaster. So the number of linkage groups in this fly is 4. In maize, the linkage groups are 10 since the haploid number is 10.

Chromosome maps have been prepared (e.g. in *Drosophila* and maize) after performing numerous test crosses involving different genes in a chromosome. The data are analysed and the results are correlated on the basis of overlapping loci involved. In this way, the sequence of known genes is established in the form of chromosome maps. When new genes are noticed, maps are revised and these genes are assigned their positions in the maps.

# Importance of Chromosome Maps

Academically, their importance lies in giving evidence as to the arrangement of genes in chromosomes, i.e. structural systems of higher order. They are useful to breeders. When a breeder desires to introduce a particular combination of genes into certain plants or animals, he has to take into consideration linkage and crossing over, because linkage may affect to a

large extent, the probability of getting a given combination out of a population of segregants. Geneticist can predict the probabilities of getting such genotypes by studying the chromosome maps, and accordingly plan his programme and estimate the extent of experimental population necessary.

## Significance of Crossing Over

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If there were no crossing over, two linked genes would have been permanently attached. If one of them was a harmful or undesirable gene and the other a useful one, there would have been no change to get rid of the harmful gene. The phenomenon of crossing over increases the pool of variation necessary for evolution. It permits genes situated on the same chromosome to take part in shuffling and reassortment and thus gives rise to new combinations. Thus, it offers the evolutionary advantages of sexual reproduction to genes in the same chromosome.

#### SUMMARY

- 1. Morgan discovered that when two genes are in the same chromosome, they tend to stay together in inheritance. This process is known as linkage. The degree or strength of linkage depends on the distance between the linked genes in the chromosome. This fact is the basis for construction of genetical or linkage maps of chromosomes.
- 2. In the case of inheritance of body colour (grey against black) and size of wings (normal against vestigial) in Drosophila, the cross between grey normal female and black vestigial male was very much different from the typical test cross 1:1:1:1. Morgan and his students explained these results by formulating the hypothesis of crossing over. This process involves an exchange of segments between the homologous chromosomes during meiosis. Because of crossing over, grey vestigial and black normal are formed. These are the new combinations of linked genes, called recombinants, and the process, recombination.
- 3. Crossing over takes place at the four-stranded stage. This is directly proved in the case of Neurospora and indirectly in Drosophila. The cytological proof of crossing over was given by Kurt Stern by using aberrent X-chromosome strains of Drosophila flies. There may be double and triple cross overs.
- 4. For the construction of chromosome maps, the three-point test cross method is employed. The proportion of double cross-overs is actually less than the expected amount. This is due to interference since the occurrence of one cross over apparently interferes with the occurrence of other cross overs in adjacent regions. The observed number of double cross overs divided by the expected number is known as the coefficient of coincidence.
- 5. Although crossing over has been reported in most of the plants and animals studied, there are some exceptions. Crossing over is absent in male Drosophila and the female
- 6. Each gene is the number of some one linkage group consisting of a number of genes held together by the physical continuity of the chromosome. A breeder has to take

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into consideration linkage and crossing over while planning his experiments. A knowledge of linkage groups and chromosome maps is useful in his work.

7. The process of crossing over permits genes situated on the same chromosome to take part in shuffling and reassortment and thus give rise to new combinations.

#### PROBLEMS

P.1 In maize, three dominant genes C, D and E are necessary for the coloured seed. The genotypes C/-D/-E/-D are coloured and all other genotypes are colourless. When the plant with coloured seeds was crossed with three tester plants, the following results were obtained:

Cross with c/c d/d E/E gave 48% coloured seed. Cross with C/C d/d e/e gave 22% coloured seed. Cross with c/c D/D e/e gave 46% coloured seed. Determine the genotype of the plant.

P.2 In plant A, the dominant gene C responsible for white corolla is at the locus 45.0 in a chromosome map. The recessive gene d for gene calyx is at the locus 50.0. Determine the number and kinds of off-spring that will be obtained in the following cross.

$$\frac{+}{C}$$
  $\frac{d}{+}$   $\times$   $\frac{+}{+}$   $\frac{d}{d}$ 

P.3 The following progeny was obtained from the cross

$$\frac{+ b + a b + c}{a + c} \times \frac{a + b + a b c}{a + b + a b c} \times \frac{a + b + a b c}{a + b + a b c} \times \frac{45}{a + c} \times \frac{a + c}{a b c} \times \frac{95}{a b c}$$

$$\frac{+b+|abc|}{abc} \times \frac{345}{a + c} \times \frac{a++|abc|}{a+c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + b + c}{a + b + a b + c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + b + c}{a + b + c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + b + c}{a + b + c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + b + c}{a + b + c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + c}{a + c} \times \frac{a + c}{a + c} \times \frac{a + c}{a + c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + c}{a + c} \times \frac{a + c}{a + c} \times \frac{a + c}{a + c} \times \frac{95}{a + c}$$

$$\frac{a + c}{a + c} \times \frac{a + c}{a + c$$

Construct the map of the genes a, b and c and find out the coefficient of coincidence.

P.4 In the test cross,

$$\frac{(+ + +)}{(e \quad f \quad g)} \times \frac{(e \quad f \quad g)}{(e \quad f \quad g)}$$

The order of the genes in parentheses is unknown.

$$(e+g)/(efg)$$
 25  $(+fg)/(efg)$  80  
 $(e f g)/(efg)$  390  $(e++)/(efg)$  85  
 $(ef+)/(efg)$  3  $(+++)/(efg)$  385  
 $(+f+)/(efg)$  30  $(++g)/(efg)$  2

1000

Determine the order of the genes and construct their map.

- P.5 In the cross  $(cde)/(+++)\times(cde)/(cde)$ , the classes of offspring with least numbers were (cd+)/(cde)and (++e)/(cde). The order of genes in parenthesis is unknown. What is their order?
- P.6 In Drosophila, there is no crossing over in the male. The map distance between m and n genes is 15 cross over units. Find out the proportion of different types of offspring and their genotypes in the following cross.

- P.7 Suppose there are three alleles +|a|, +|b| and +|c| in Drosophila. The genes a, b and c are recessive and sex-linked. They are situated in the X-chromosome and their order is a-b-c. The map distance between a and b is 10 units and between b and c is 15 units. The coefficient of coincidence is 0.4. If the female of the genotype +++|abc| is crossed with the wild type of male, how many types of genotypes will be obtained, and what will be their frequency among 1000 offspring?
- P.8 Suppose the proportion of crossing over in both the sexes in a ruminant animal is same and their ton live been a par gam amosomoulo chromosome map is as follows:

The genotype ddE is brown, D|-E| is black, and D| - ee or ddee is white. F| is of long ears and fof short ears. The coefficient of coincidence is 2/5.

(i) Determine the genotypes and phenotypes and their number in the cross

$$\frac{D \ E \ F}{d \ e \ f} \times \frac{d \ e \ f}{d \ e \ f}$$

The total progeny is 1000.

ading a test country with the distribution of (ii) Find out genotypes and phenotypes and their proportions in the following cross.

$$\frac{D}{d} = \frac{E}{e} = \frac{D_{1}}{e} = \frac{E}{e}$$

I when are two alleles of the same age and the prompts to the prompts and a many

# FURTHER READING

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# 19. Multiple Alleles

We have already seen that genes exist in two different alternative forms or alleles which occupy the same locus in a chromosome. Because one of the alleles has arisen from the other by mutation, the former is the mutant gene and the latter the normal or wild type gene. A careful examination of many gene loci has indicated that the same gene may possess many mutant forms. They are identified as alleles since they occupy the same locus on the chromosome map. So a gene A will not only mutate to a but may also give rise to othe stable alleles like  $a^1$ ,  $a^2$ ,  $a^3$ , etc. Such alleles are called multiple alleles. The relation between alleles which are members of a multiple series is essentially the same as in the case of a simple pair of alleles. However, some additional methods and ideas have been developed to detect multiple alleles to represent a large variety of genotypes which they produce and to study the phenotypic relationship among the alleles within a series.

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Multiple alleles possess certain characteristics. These are given below.

- 1. Multiple alleles reside always at the same locus in the same chromosome.
- 2. No crossing over occurs between alleles of multiple allelic series. Hence, when two alleles are involved in a cross, they are recovered in the  $F_1$  or test cross progeny.
- 3. Multiple alleles always affect a similar character. This fact points out that they have a similar origin. In *Drosophila*, all the alleles of the white eye locus affect eye colour.
- 4. The wild type allele is usually dominant. The other alleles of the series may show dominance or cause intermediate phenotypic effects when two different alleles companies together in the same genotype.
- 5. When any two alleles of the same series are crossed, the phenotype is of a mutan character.

When it is suspected that given genes may be alleles and if they do not reveal any one c the above characteristics, they are not probably alleles.

Multiple alleles have been used by geneticists for analysing the nature of genes. It has been observed that some multiple alleles are really more complex than they were thought to be at first.

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# Examples of Multiple Alleles

## White Eye Series in Drosophila

This is a classical example of multiple alleles. The wild type gene (W and  $w^+$ ) causes red ey

It is the normal for all the genes affecting eye colour. This gene not only mutates to a  $g^{e_1}$ 

causing white eye but also to a variety of intermediate forms known as apricot, blood, buff, coral, honey, pearl, etc. on the basis of similarity of colour of these materials. These alleles are capable of mutating to one another and also to white or red. More than 15 alleles have been identified at this locus. It is likely that this number would be more, if finer gradations of colour could be recognised.

There is a definite procedure for designating multiple allelic series, and the various forms are indicated by superscripts. For example, in the case of white eye series in Drosophila, W or w+ stands for the wild type red while wa indicates apricot, wb blood and w white.

### Coat Colour in Rabbit

In case of the wild type of rabbit, the coat colour is brownish grey which is the normal colour. There are several mutant coat colour phenotypes, e.g. chinchilla, Himalayan and albino. Their existence is explained on the basis of multiple allelism.

If a homozygous chinchilla (full coloured) is mated with an albino, all chinchillas are obtained in the  $F_1$  generation. In the  $F_2$  generation, however, the typical ratio of 3 chinchilla: 1 albino is obtained. These results indicate that chinchilla is dominant to albino and

both the genes are allelic to each other.

The Himalayan rabbit has a white fur except at the extremities like ear, nose, tips of feet and tail which are pigmented. If a chinchilla rabbit is crossed with a Himalayan rabbit, the  $F_1$  yields all chinchillas while in the  $F_2$  the typical ratio of 3 chinchilla: 1 Himalayan is obtained. This means that the full colour is due to a dominant gene and the Himalayan due to its recessive allele. It will be interesting to know the results of a cross between a Himalayan rabbit and an albino one. In this cross, all the F1 progeny is Himalayan and in the  $F_2$  again a 3: 1 ratio is obtained, Himalayans being 3 and albino 1. So, Himalayan and albino genes are allelic to each other.

The above results indicate clearly that the genes concerned with coat colour in the rabbit represent a series of multiple alleles. Each mutant allele produces a different coat colour. It may be that each allele affects synthesis of pigment in a different way. In other words, each allele is derived by a mutation which affects the function in a different way.

Let us now represent the genotypes and phenotypes on the basis of the results of the above mentioned crosses.

| cc         | THE PARTY AND TH | cehceh    | Chinchilla            |
|------------|--|-----------|-----------------------|
| Ccch       | and ad season if the   | cchch     | The 100 to 110 - 10 c |
| Cch        | Full colour  | cchc 1    | Duro, but not bell    |
| Cc         | has lifered to   | chch .    | Himalayan             |
| cei-Chinch | nilla  | c*c       | Luco otto oud         |
| TOT SHE    | main a man   | cc-Albino | and desired the p     |

It is said that the original wild type of rabbit was grey in colour. It must have been because of the gene CC. At some time mutation had occurred in one of the C, about which we know very little. This caused a change in the chemical composition of the gene in such a manner that it became incapable of producing the colour. Let us represent this mutant gene

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as c. When the gamete containing this gene unites with the gamete possession gene C, the resulting individual will be of the genotype Cc. The phenotype of this heterozygote will be, however, the same as of CC. When this  $F_1$  will form the gametes, half will contain C and half c. So an individual formed as a result of a union of two gametes each having c will be an albino with the genotype cc. It might have happened during evolution that one of the genes CC of the rabbit mutated in such a way that it restricted the development of colour only at the extremities like the ear, nose, tail and tips of feet. We designate this gene as  $c^{ch}$ . A Himalayan of the rabbit must have been formed by the union of gametes of the constitution  $c^h$ . Finally, when there was a cross between the Himalayan and the albino,  $c^h$  and c must have behaved as alleles.

#### Multiple Alleles in Maize

A multiple allelic series affecting seed colour in maize has been observed. The normal seed colour is purple due to the purple pigment occurring in the aleurone layer. Let us represent the gene responsible for this normal purple colour by A. A mutation caused it to change to a, resulting in white seed. Another recessive mutation changed ++ to a so as to form light purple seed. The mutants a and a' also affect other parts of the plant.

#### Multiple Alleles in Cotton

In cotton, gene L is responsible for narrow leaf while its mutant allele l is responsible for broad leaf. Since they occupy the same locus, they segregate in a 3:1 ratio in a cross. If gene l mutates to forms in different cases, there will be variations in the same character and a multiple allelic series will arise. In fact, such is the case in cotton. l has mutated to  $l^B$  (mutant broad),  $l^I$  (lacinated) and  $l^I$  (mutant intermediate). These different grades of lobing have arisen due to the mutation of the single gene L and hence all mutant genes are alleles of each other.

## Detection of the Number of Alleles in a Series

To find out the number of alleles at most loci is a difficult task as it is limited by the inability of the observer to recognise finer differences. However, if we want to determine the number of possible alleles, it must be in a system in which any two alleles can be identified as being the same or different without any doubt. It may be that a large number of mutant alleles occur in nature, but not being persistent for a long time so as to be identified, we are unable to detect them. Mutant genes are generally harmful and are, therefore, eliminated by natural selection. We therefore, require a system where new mutants are retained rather than eliminated.

There occur self-sterility (self-incompatibility) genes in many plants. They form an ideal system for our purpose. These genes inhibit the pollen from fertilising the ovule if it falls on the pistil of the same plant. A pollen grain containing a specific allele will not succeed in bringing out fertilisation if it falls on a plant possessing the same allele. For instance, a pollen grain with the allele  $s^1$  is unable to fertilise a plant of the genotype  $s^1/s^2$  or  $s^{1/3}$  but is able to fertilise one with  $s^2/s^3$  genotype. This system is ideal for the study of multiple alleles.

eathers now

If an allele does not allow the pollen from functioning, it is the same as one of the two alleles in the female plant, if not, it is different.

This system offers another advantage. If an allele is very common, pollen grains which carry it will often fall on flowers carrying the same allele and thus will remain non-functional. So these genes will have a tendency to decrease in the population. Similarly, pollen grains carrying a very rare gene will have an excellent chance of fertilising a flower successfully. Hence, the rare genes will have a tendency to increase. As common genes decrease and rare genes increase, the tendency will be towards a frequency exhibiting intermediate equilibrium. Therefore, every mutant occurring in this system tends to be retained in the population rather than be eliminated. In the case of evening primrose plants, 37 different sterility alleles in a population of about 500 have been reported. In red clover, more than 200 alleles have been identified.

## Action of Mutant Gene in Terms of Normal Allele

We usually express the relationship between alleles in terms of dominance. For example,  $A_1$  is dominant over  $A_2$ , if  $A_1A_1$  and  $A_2A_2$  are indistinguishable. If  $A_1A_2$  is mid-way between  $A_1A_1$  and  $A_2A_2$ , there is no dominance. If  $A_1A_2$  is nearer to  $A_1A_1$ , we say that  $A_1$  is partially dominant. There are examples where  $A_1A_2$  is out of the range of two homozygotes. This is the case of overdominance. In most of the cases studied, the wild type allele is nearly but not completely dominant. For example, mutants which act as lethals in homozygous condition usually reduce heterozygotes by about 5%.

Let us again turn to the white eye locus in *Drosophila*. Both the alleles w<sup>+</sup> and w<sup>a</sup> form the pigment, but the former is more active. It may be also possible that w and w<sup>a</sup> alleles are actively engaged in reducing the amount of pigment. Müller (1939) devised techniques to distinguish between these and other possibilities. He introduced appropriate terms for describing the phenotypic effect of an allele in terms of that of the wild type allele.

If a mutant allele does not produce a detectable effect, it is called an *amorph*. For example, white eye allele w is an amorph. An allele which acts in the same direction as the normal allele but less effectively is termed as a *hypomorph*. The apricot mutant  $w^a/w^a$  is more normal (since more eye pigment is produced) than w/w. Since w is amorphic,  $w^a$  must be acting in the direction of the normal allele.

$$\frac{A}{A} \rightarrow \frac{A}{\text{deletion}} \rightarrow \frac{A}{+} + \frac{+}{+}$$

A mutant with activity in a direction opposite to that of the normal allele is called an antimorph. For example, the mutant, abnormal abdomen (A).

The symbol A indicates 'more abnormal than'. It may be that a mutant gene is acting in the same direction as the normal allele but it appears as though it is acting in the opposite direction.

Müller has also suggested two possibilities. According to him, a hypermorph is more active than a normal allele. Most of the mutants studied are found to be either hypomorphs or amorphs. Hence, it seems that the common result of mutation is partial or complete activation of the gene rather than a change to a new function.

#### Isoalleles

The alleles which at first appear to be similar but after testing prove to be different are called *isoalleles*. Isoalleles were first identified by Stern and Schaffener (1943). The detection of the number of isoalleles depends upon the number of phenotypic criteria used to compare alleles and how small a phenotypic criterion used to compare alleles and how small a phenotypic difference can be perceived. There is extensive work on the blood type isoalleles and isoalleles in *Drosophila*.

A person belonging to blood group A may have one of the three different subtypes  $I^A$ ,  $I^{A_1}$ ,  $I^{A_2}$  and  $I^{A_3}$  which have resulted from differentiation of  $I^A$ . Three slightly different allelic alternatives have also been reported for  $I^B$ . At first these alleles appear similar but after testing proved to be different. Therefore, these are isoalleles. There are several other examples of isoalleles. These have been identified on the basis of their response to temperature, humidity or to agents that modify mutation rates in the presence of non-allelic genes.

#### **Pseudoalleles**

Two genes are sometimes so closely linked that they are separable only by a rare crossing over. Such genes are called *pseudoalleles* since they are likely to be considered as alleles of a single gene.

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Lewis (1951) demonstrated the presence of pseudoalleles in *Drosophila*. This is known as the 'star-asteroid case'. He obtained a recessive mutation producing a rough eye in the homozygous condition. It was at locus 1.3 in the chromosome II. This was the identical location of the gene Star, which was a dominant mutation. It also affected the eye which became rough and had a slight glum and so named Star, the gene symbol being s.

If we apply the criteria of alleles that these genes have the same location in the chromosome and have similar function, they should be considered as alleles. As no crossing over was observed, this conclusion was strengthened. Crosses between Star and Star-recessive also produced about 1 fly as a recombinant in a population of 5000 flies, i.e. there was 0.02% recombination. Hence the Star-recessive was then called 'asteroid' with the gene symbol ast. So what were assumed as alleles were proved to be actually pseudoalleles. Two different kinds of heterozygotes could be produced as indicated below:

(i) s+ast/s ast and (ii) s ast/s+ ast+

#### **Position Effect**

It was observed surprisingly that although these two kinds of heterozygotes had the same alleles, they exhibited different phenotypes when the alleles were in different combinations. The first heterozygote was found to have a very much reduced eye while the second heterozygote was with normal eye. It means that when the position of alleles in the chromosomes is changed, the phenotype is affected. Such an effect is called the position effect. In this

case, the phenotype is not determined by the total number of alleles present, i.e. genotype but by their position in the chromosomes. If the fly possesses two normal alleles in the same chromosome and two mutant alleles in the homologue, a normal eye will be formed.

The first conclusive proof of the position effect was given in *Drosophila melanogaster*. It concerns the mutation bar eye. This was recognised as a dominant locus at 57.0 on the genetic map of the X-chromosome. The gene causes reduction in the number of facets of the compound eye, so that the eye looks like a narrow band or bar. Zelang observed that in a culture homozygous for bar, the 'mutations' occurred back to the wild type (normal eye) and to a very much extremely reduced type of eye (very narrow) called 'ultra bar'. The nature of the extraordinary mutations occurring at the bar locus was first explained by Sturtevant. He found that the bar chromosome contained a duplication for a short section. The ultra bar section had this section in triplicate while the normal chromosome in a single dose. Therefore, it was concluded that the original change which had produced the mutation bar eye from the normal was a duplication of a short section of the chromosome due to unequal crossing over (Fig. 19.1).

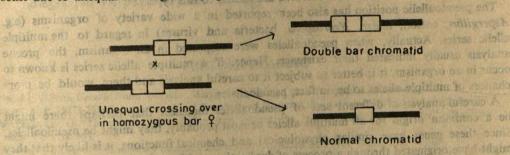
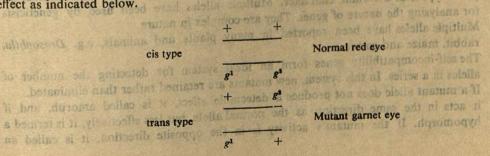


Fig. 19.1 Formation of double bar due to unequal crossing over in Drosophila. For convenience, only two chromatids are drawn in the process of crossing over.

When the mutant alleles are situated in one chromosome and the two 'normal' alleles in the other  $\left[\frac{A}{a} \frac{B}{b}\right]$ , the arrangement is called *cis* and the alternative form  $\left[\frac{A}{a} \frac{b}{B}\right]$  as the *trans*-arrangement. If this results in different phenotypes, the position effect is called *cis*-trans position effect.

Two mutant genes  $g^1$  and  $g^2$  in *Drosophila* producing garnet eye colour are typical. The crossover tests indicate that  $g^1$  is to the left of  $g^2$ . The genes show the cis-trans position effect as indicated below.



These results can be explained by regarding the entire region as a single gene and assuming  $g^1$  and  $g^2$  as two sites where mutational changes had taken place. In the upper diagram, the phenotype is shown as the normal red eye since there is one intact normal gene which produces a normal phenotype because of its dominance. In the lower diagram, neither gene is normal and hence the normal phenotype did not result.

When a gene changes its position from a euchromatic region to another euchromatic region, the effect is stable and known as the stable or S-type position effect. These effects are characterised by a uniform change of action of the normal gene. The bar eye position effect comes under this type. When the gene changes its position from the euchromatic region to the heterochromatic region or vice versa, the result is a variegated or mottled type of phenotype. Hence, such a change is called as variegated or V-type of position effect. In this case, there is somatic instability of the gene action. Of such a position effect in Drosophila, there is the example of the effect of chromosomal rearrangement in regard to white eye locus. Heterozygotes which have gene w on the normal chromosome and its allele w<sup>+</sup> on a rearranged chromosome exhibit a variegated pattern in the colour of eye. The position effects recorded in Oenothera are similar to this type.

The pseudoallelic position has also been reported in a wide variety of organisms (e.g. Aspergillus, cotton, maize, Neurospora, bacteria and viruses) in regard to the multiple allelic series. Actually, when pseudoalleles were suspected in an organism, the precise analysis usually indicated their existence. Hence, if a multiple allelic series is known to occur in an organism, it is better to subject it to careful analysis and there would be more chances of multiple alleles to be, in fact, pseudoalleles.

A careful analysis of different sets of pseudoalleles indicates that perhaps there might be a common origin for all multiple alleles or most probably, they might be pseudoalleles. Since these genes have similar physiological and chemical functions, it is likely that they might have originated through a process of duplication making mistakes in crossing over.

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- 1. A gene may exist in more than two forms. One of the alleles is the normal or wild type of gene, the others have arisen by mutation. They occupy the same locus on the chromosome map. Such alleles are called multiple alleles.
- 2. No crossing over occurs between alleles of the multiple allelic series. They always affect a similar character. When any two alleles of the same series are crossed, the phenotype is of a mutant character. Multiple alleles have been used by geneticists for analysing the nature of genes. They are complex in nature.
- 3. Multiple alleles have been reported in many plants and animals, e.g. Drosophila, rabbit, maize and cotton.
- 4. The self-incompatibility genes form an ideal system for detecting the number of alleles in a series. In this system, new mutants are retained rather than eliminated.
- 5. If a mutant allele does not produce a detectable effect, it is called amorph, and if it acts in the same direction as the normal allele, but less effectively, it is termed a hypomorph. If the mutant's activity is in the opposite direction, it is called an

- antimorph. A hypermorph is more active than the normal allele while a neomorph displays an activity quantitatively different from that of the normal allele.
- 6. The alleles which at first appear to be similar but after testing prove to be different are known as isoalleles. Pseudoalleles are two genes so closely linked that they are separable only by a rare crossing over. The star-asteroid case in Drosophila is an example of pseudoalleles.
- 7. When the position of alleles in the chromosome is changed, the phenotype is affected and the effect is called the position effect. When the mutant alleles are situated in one chromosome and the two 'normal' alleles in the other, the arrangement is called the cis and the alternative form the trans arrangement. If this results in different phenotypes, the position effect is termed cis-trans position effect. When a gene changes its position from a euchromatic region to another euchromatic region, the effect is known as stable or S-type position effect. When a gene changes its position from the euchromatic region to the heterochromatic region or vice versa, there is variegated or V-type position effect. care of measurement. They are therefore to be expressed in profession as our keeping

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# 20. Quantitative Inheritance

So far, we have studied the inheritance where differences between various characters can be observed with the naked eye, for example, tall vs. dwarf plant, coloured vs. white flowers. These are sharply contrasting alternate characters of individual plants and are known as qualitative characters and their inheritance is called qualitative inheritance. Mendel's work comes under this type of inheritance. However, there are many characters whose differences are not obvious. They exhibit differences of degree along a continuous scale of measurement. They are, therefore, to be expressed in units such as cm, kg or litre. When there is a large number of individuals in population and such characters are taken into consideration, the recorded values do not fall into groups since the variation is continuous, for example, yield of crop and intelligence. Such characters are called quantitative characters and their inheritance as quantitative inheritance. The identification of these characters does not depend on one or two major genes. The action and interaction of several genes are involved in their expression. The phenotype in such cases is also influenced considerably by environment.

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The study of inheritance of quantitative characters started in the first decade of the twentieth century. Some controversies arose during this period. One of the undecided important problem was the application of Mendelian laws in case of inheritance of quantitative characters. The question was whether a Mendelian interpretation of inheritance could be applied to all characters or only to those quantitative characters which exhibit a clear cut segregation for distinct phenotypes in the segregating generation. It was observed in certain cases that the  $F_1$  generation was more or less intermediate between two parents and there was great segregation in the  $F_2$  generation without any division into sharp discrete classes. How are these characters inherited? Are these inherited in the Mendelian fashion? Such were the questions in the minds of geneticists before 1910.

East was primarily responsible for pioneering the work on the inheritance of quantitative characters. He was at the Bessey Institution of Harvard University, USA. During the same time, the Swedish geneticist, Nilsson-Ehle was engaged in developing what is referred to as the multigene hypothesis in cereals. He discovered two genes responsible for the yellow endosperm in maize. He called them  $Y_1$  and  $Y_2$ . He observed from a plant heterozygous for both these genes  $(Y_1/Y_1, Y_2/Y_2)$ , that the progeny were in the ratio of 15 yellow: 1 white. Similar results were obtained by Nilsson-Ehle in wheat. East thought that in such cases, a large number of genes might be responsible for the characters like yield of crop, milk yield or height of the plant or animal. When hybridization occurred, the cumulative effect of these genes was expressed by increase in the dimension of the concerned character. This was indeed a new concept in the field of genetics and came to be known as the

multiple gene hypothesis. However, even in the inheritance of quantitative characters, East recognised the Mendelian basis though an individual character might be affected by several or many genes.

# Multiple Gene Hypothesis and the law of the restaurance of engineer fulfilles and

We owe this hypothesis to East (1910) and Nilsson-Ehle (1908-11). It represents one of the significant advances in genetics. It was put forward around 1910. Since then, it has been modified and expanded by a large number of workers. However, its basic tenets stand as corner-stones in our understanding of quantitative inheritance.

Let us consider the multiple gene hypothesis in its simplest form. According to it, many aspects of quantitative inheritance can be explained on the basis of action and segregation of a number of allelic pairs having duplicate and cumulative effects without complete dominance. Let us take a hypothetical example to make this clear.

# Hypothetical Example of Quantitative Inheritance

The quantitative character involved is the ear length. Let us assume the following:

- 1. There are two true breeding parent plants, one possessing relatively long ears and other relatively short ears. A cross is made between these two parents.
- 2. It is supposed that under the experimental conditions, environment is uniform and hence, it is not responsible for variation in ear length.
- 3. Genetic differences for ear length in parent plants are due to three independent genes. The parent plant for long ear is homozygous for allelic forms represented by capital letters AA/BB/CC. The parent for short ear is homozygous for allelic forms designated by small letters aa/bb/cc.
- 4. The alleles represented by small letters are inert in regard to ear length.
- 5. The action of each allele represented by a capital letter is such that it contributes one centimetre of ear length of any plant in which it occurs.
- 6. Each of the parents possesses the same genotype for ear length. It is expressed by 10 cm of growth. This is besides from genes represented by A, B and C.
- 7. If the genotype of short ear parent is aa/bb/cc, the length of the ear will be 10 cm while that of the long parent 16 cm since each gene represented by a capital letter contributes an increase of one cm, i.e. AA = 2 cm. BB = 2 cm, and CC = 2 cm (total 6 cm). Therefore the ear length is 10+6=16 cm. the state of the same of the state of the st If these two parents are crossed, down the min that the AA BB CC x aa bb cc somey the part of both a condition which the dear of

Santa sonaire for a trate of F1: Aa Bh Cc Salans Jahr to space the destroy alderale stone The ear length of the  $F_1$  hybrid will be 10+3=13 cm. This is exactly intermediate between the parents. In case of  $F_2$  also, the mean car length will be 13 cm, i.e. same as that of  $F_1$ , but the  $F_2$  population will exhibit considerable variation in ear length because of the effect of segregation. This will be clear from Table 20.1.

Table 20.1 A hypothetical example of inheritance of ear length to illustrate the multiple gene hypothesis to an arrange as a basing open and veral or many ger and

Individual contribution of genes are supposed to be

A=B=C=1 cm a=b=c=0 cm

The residual genotype is responsible for 10 cm ear length. Parent with long ears × Parent with short ears

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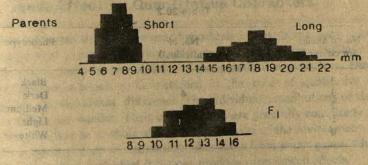
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Several of the results which we have predicted on the basis of our above hypothetical experiment conform to the actual findings of Emerson and East (1913) in regard to the inheritance of ear length in maize. They also observed that the ear length of the F2 was approximately same as that of the  $F_1$ . But the  $F_2$  showed greater variability and the extreme measurements in  $F_2$  overlapped well into the distributions of parental values. When you find that the  $F_1$  is intermediate,  $F_1$  and  $F_2$  mean values are approximately equal and  $F_2$  much more variable, you should suspect that you are dealing with quantitative inheritance, since these are the characteristics of it (Fig. 20.1). Figure 20.1 gives data obtained by Emerson and East in the form of histograms. It is on the basis of these data that they arrived at the above mentioned conclusions.

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cas are cougliy examined, it is reduced Fig. 20.1 diograms of distribution of ear length in maize in the parental lines,  $F_1$  and  $F_2$  generations studied by Emerson

# Skin Colour Inheritance in Man

Davenport (G.C.) and Davenport (C.B.) (1910) and Davenport (C.B.) (1913) worked out the skin colour inheritance in the progeny of Negro-White matings. It seem that their work had a deep impact on the thinking of East. The work pointed out that there are two pairs of genes responsible for the differences in the pigment between Negroes and Caucasian people.

C.B. Davenport was one of the pioneers who had studied the problem of skin colour of people of Jamaica and Muda. There were frequent intermarriages between Negroes and White people. His study made it possible to detect different degrees of pigmentation. He observed that when a Negro (AB|AB) and a White (ab|ab) were crossed, the  $F_1$  hybrid (called mulatto) was AaBb. The colour of mulatto was intermediate between the black and white parent. However, the F2 progeny were surprising. Some were dark as the black parent while most of the offspring had their colour somewhat in between the two parents and there was a child who was of much lighter colour than the rest. This last child exhibited many other characteristics of the Negro or rather of both the Negro and White races. As regards the child's skin colour, it was almost as light as that of a White. These results are in agreement with those involving quantitative inheritance. They are also based on Mendelian principles. In the present case, the genotype of the hybrid mulatto is AaBb. It is heterozygote at two loci and so like the other dihybrid, it produces four types of sperms of egg cells: AB, Ab, aB, and ab. Therefore, when mating takes place between two mulattoes, 16 combinations can be formed by their egg or sperm cells. Table 20.2 summarises the results of such matings.

The number of individuals having any given number of the alleles for colour can be obtained by expanding the binomial as follows:

$$(a+b)^2 = 1a^4b^0 + 4a^3b^1 + 6a^2b^2 + a^1b^3 + 1a^0b^4$$

a represents alleles for colour with the exponent standing for the number of such alleles. The coefficient of each term represents the number of individuals.

| -   | 100 |   | ^ | - |
|-----|-----|---|---|---|
| Ta  | 10  |   | • |   |
| ı a | 16  | - | • |   |

| No. of alleles | No. of individuals | Phenotype                        |
|----------------|--------------------|----------------------------------|
| 4<br>3<br>2    | 1<br>4<br>6        | Black<br>Dark<br>Medium<br>Light |
| 1 0            | 4                  | Ligh<br>Whi                      |

Thus, we have seen that the skin colour in man is a quantitative character. Unfortunately, the number of genes actually involved in its inheritance is not yet known. It should, however, be borne in mind that Davenport's model based on two pairs is too simple to account for the available data. Even if the offspring of the  $F_1$  mulattoes are actually examined, it is a difficult task to assign the progeny into definite colour classes and to count the number of each class. There is a gradation of colour with the lightest and intermediate shades.

# Inheritance of Flower Length in Tobacco

The data obtained in this study are diagrammed as histograms in Fig. 20.2. You will observe that there is considerable variability which is due to environment or residual heredity. This is shown by the spread in the parent and the  $F_1$  populations. It will be seen that the  $F_2$  is more variable in length of flower than the  $F_1$  but none of the individuals in the former generation is as extreme as the parent lines. In this study, several hundred  $F_2$  flowers were measured. It indicates that a large number of genes, at least a dozen must have been involved in this case. If some genes are dominant or if they do not all produce an equal effect, then it is likely that many times the number of genes may be more than a dozen.

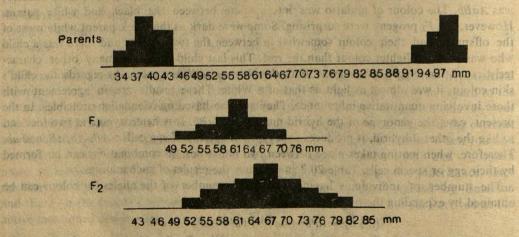


Fig. 20.2 Idiograms of distribution of flower length in tobacco in the parents,  $F_1$  and  $F_2$  generations.

# Nature of Genes Effecting Quantitative Characters

We have already seen what the multiple gene hypothesis means. It is a good working hypothesis. So it can be used to explain the findings in cases of quantitative inheritance. According to this hypothesis also, genes are responsible for the quantitative characters. These genes are very much like those genes which we have studied in the case of qualitative inheritance. There is, however, a significant difference. The individual contributions of these genes to phenotypic differences are smaller. Their contributions are generally completely masked by the effects of the genotype as a whole and by the influences of the environment. Mather has designated such genes as polygenes. He distinguishes them from major genes which can be easily recognised since their individual functions produce pronounced effects.

Though a terminological distinction is made between 'multiple genes', it does not imply that there is no overlapping of these two types or no area between them. is applied to the property of the company of the property of t

## will at care to explained very well on the basis of this to prognets) Yes or the lifting board, the m Duplication Effects of Major Genes and Multiple Gene Systems ologic samp strip amonth aviilbis

There may be duplication of the phenotypic effect which is generally regarded as due to the operation of specific multiple gene complex or the action of a single gene. For example, the normal continuous range of stature in man appears to be due to multiple gene effects. L'EN STERVINSORIES CE AMBORRE LE CHIESTORIES AND PROPERTY COMPLEMENTATION OF A REPORT OF THE PROPERTY OF THE P

# organization of the modern of the modern of the state of Role of Major Genes in a Multiple Gene System

It may be asked whether there are genes which can serve in a dual capacity by simultaneously affecting both the qualitative and quantitative characters. The present evidence indicates that there are such genes. For instance, in white clover, there are two independent dominant genes whose interaction results in mottling and lesions of the leaf blades which are normally smooth green. Besides this qualitative change, there is also a significant effect due to the dosage of dominant genes on the number of leaves, which is a quantitative character.

# Linkage and Multiple Gene Complex

It is very difficult to determine linkage relationships between members of multiple-gene complex. This is because individual genes usually do not produce identifiable phenotypic effects. However, several examples are now known where linkage between major genes and multiple genes is well established.

# Additive Effect and Location of Multiple Genes

The inheritance of quantitative characters is a complex phenomenon. It is controlled by a large number of factors and there are also differences in the effects of the individual genes. Some genes may affect the character in the plus direction while there may be genes working in the minus direction. The individual effects of these genes may not be equal but their total effect remains additive. It is also found that a particular character may be controlled by multiple factors or by a single factor.

As regards location of genes, all of them may not be situated in the same chromosome. They may occur in different chromosomes. For example, in *Datura*, the genes controlling plant height are situated in all the 12 chromosomes.

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# Multiple Gene System and Gene Action

The multiple gene hypothesis has been found to be a good working hypothesis in many cases. Yet it suffers from one drawback. It assumes simple additive effects of the action of all the operative genes. It is true that the results of Nilsson-Ehle's experiments on kernel colour in wheat can be explained very well on the basis of this hypothesis. Yet on the other hand, there are many cases of quantitative inheritance which cannot be explained on the basis of simple additive effects of the genes involved.

A good many studies of quantitative inheritance point out that gene substitution may have geometric rather than arithmetic (additive) effects. In such cases, the genes appear to contribute their effects not by adding or substracting constant amounts but rather by multiplying or dividing the residual genotype effect by some constant amount. The mean value of a quantitative character in  $F_1$  in such cases is more nearly approximate to the geometric mean rather than to the arithmetic mean. The arithmetic mean of two different numbers is midway between them on an additive scale. For example, the arithmetic mean of 2 and 8 is 5, which is midway between 2 and 8, and the difference between successive terms (2, 5, 8) is three. On the other hand, the geometric mean, although a similar midpoint, is on a multiplying (geometric) scale. So the geometric mean of 2 and 8 is 4 which is the term between 2 and 8 in a geometric series (2, 4, 8). In this case, there is a constant of multiplication. That is, the geometric mean of two numbers is the square-root of their product.

MacArthur and Butler (1938) studied inheritance of fruit weight in different tomato crosses. The results obtained by them are summarised in Table 20.3.

Table 20.3 Mean fruit weight in grams of different tomato crosses (after MacArthur and Butler, Genetics 23: 254, 1938)

| Large parent Smaller parent       | Large | Small Small | . F1    | Geometric     | Arithmetic   |
|-----------------------------------|-------|-------------|---------|---------------|--------------|
| and loved that the of pict        | P     | P           | (pubry) | nel mean      | and mean     |
| Parents differing greatly in size |       | TO BOTH     | asignes | s terover you | iswoH amenia |
| Large pear × Red currant          | 54.1  | 1.1         | 7.4     | 7.4           | 27.6         |
| Putman's forked × Red currant     | 57.0  | 1.1         | 7.1     | 7.7           | 29.0         |
| Honor bright × Yellow pear        | 150.0 | 12.4        | 47.5    | 43.3          | 81.2         |
|                                   | 55 黄河 | LV          | 44.0    | treet and     | Addition H   |

The above data indicate that wherever the parents differ much in size, the geometric mean of the parent values approximates closely the  $F_1$  mean values and not the arithmetric mean. Let us take the first combination from the table and assume that LP represents the Large

pear genome and RC the Red currant genome. Let us now compare the mean values, RC/RC = 1.1 g, LP/RC = 7.49 g and LP/LP = 54.1 g. These values indicate the presence of a geometric series wherein the effect of substituting each LP genome is to multiply by approximately 7. Thus, a series 1.1:7.7:53.9 is obtained, which is very close to the actual figures 1.1: 7.4: 54.1. Manual on Son of the Common of the contract of

Smith worked on the inheritance of quantitative characters in different species of Nicotiana and demonstrated convincingly that the multiple genes in this case acted geometrically. The character he studied most extensively was the length of the corolla of the flower. He determined the effect on this character by adding a single extra chromosome into different species of Nicotiana and also into the parental species. He found that the extra chromosome acted in such a way as to multiply the normal mean by a constant. In other words, it acted in a geometric fashion.

The available data on the quantitative inheritance lead to the conclusion that multiple genes can act either arithmetically or geometrically. the classic way to be a superior to the superior where to any fith a school or discount from what has been been a selection of the

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- 1. The characters which exhibit differences of degree along a continuous scale of measurement are called quantitative characters and their inheritance quantitative inheritance. The action and interaction of several genes are involved in the expression of these characters. The phenotype in such cases is also influenced considerably by environment.
- 2. The multiple gene hypothesis is due to East and Nilsson-Ehle. According to it, many aspects of quantitative inheritance can be explained on the basis of action and segregation of a number of allelic pairs having duplicate and cumulative effects without complete dominance. It is explained by giving an hypothetical example as well as skin colour inheritance in man. The individual contributions of the genes involved in the phenotypic differences are smaller. Mather calls them polygenes.
- 3. Although the multiple gene hypothesis is a good working hypothesis in many cases, it has one drawback. It assumes simple additive effects of the action of all the operative genes. A good many examples of quantitative inheritance point out that gene substitution may have geometric rather than arithmetic (additive) effects. Works on inheritance of fruit weight in different tomato crosses by MacArthur and Butler and of the length of corolla in different species of Nicotiana point out that in these cases genes act in a geometric fashion. Hence, the conclusion is that multiple genes can act either arithmetically or geometrically.

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# 21. Sex Chromosomes and Sex Linkage

## Sex Chromosomes

In 1891, a German biologist, Henking discovered that a particular nuclear structure could be located during spermatogenesis of certain insects. He found that half the sperms received this structure, while the other half did not. He did not understand the significance of this structure and called it the X-body. He also noted that some sperms were different from others because of the presence or absence of this structure. McClung (1902) verified his observations and himself made a cytological study in several species of grasshopper. He showed that the somatic cells in the male and female grasshopper contained different chromosome members (Fig. 21.1). He found X-body in the spermatogenesis but not in the

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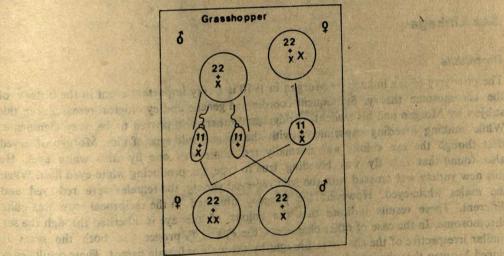


Fig. 21.1 Sex determination in grasshopper.

oogenesis of this insect. Hence, he associated the X-body with sex chromosomes but erroneously stated that it was characteristic of the male. However, he discovered an important fact that the specific chromosomes formed an unequal pair in one sex while an equal pair in the other sex. The behaviour of these chromosomes was like that of assumed sex genes and, the other sex. The behaviour of these chromosomes and the rest as autosomes. Since then, sex hence, the former were called sex chromosomes and the rest as autosomes.

chromosomes have been reported in many animals and plants. The sex with an unequal pair may be either male of female. In human beings there are 44 autosomes and 2 sex chromosomes. Since a man possesses an unequal pair of sex chromosomes, one is called the X- and the other the Y-chromosome. On the other hand, a woman has an equal pair of sex chromosomes, and so both the members of the pair are X-chromosomes. Similar is the condition in Drosophila. The male fly has an X- and Y-chromosome, while the female has two X-chromosomes. Both of them possess autosomes. The male Drosophila is heterogametic because it produces two types of gametes, one with an X- and the other with a Y-chromosome, whereas the female is homogametic as it forms only one type of gametes bearing an X- chromosome. XY type is found in different kinds of animals, e.g. Drosophila and mammals. In plants, Lychnis (Melandrium) is an example. Usually X- and Y-chromosomes differ in shape and size and can be recognised through the microscope. In mosquito, however, they are identical.

In some organisms, though the male is heterogametic, it is devoid of a Y-chromosome and hence, it produces gametes either with or without an X-chromosome. Such a condition is seen in grasshopper and *Protener* bug. In these animals, the female is XX while the male is XO. In birds, Lepidoptera, reptiles, some fishes and Amphibia, the male is homogametic.

In Hymenoptera (bees, wasps and ants), the male develops from the unfertilised egg while the female from the fertilised egg. Hence, the male is haploid and the female is diploid. Meiosis in the male is modified so that it possesses only one set of chromosomes. Different classes in bees such as queen and workers are due to differential nutrition.

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## Sex Linkage

### Drosophila

The discovery of sex linkage by Morgan in 1910 is a very important event in the history of the chromosome theory. Subsequent coordinated genetic and cytological researches in this subject by Morgan and his student, Bridges and others also proved to be very significant. While making breeding experiments with the normal wild type of flies, Morgan observed that though the eye colour was normally red, there was one fly with white eyes. He also found that this fly was breeding true to type, i.e. producing white-eyed flies. When this new variety was crossed with the wild red-eyed variety, the females were red- $\alpha$  yed and the males white-eyed. However, the progeny obtained in the reciprocal cross was quite different. These results indicate that the colour of the eye is inherited through the sex chromosome. In the case of other characters, the  $\alpha$  and  $\alpha$  progeny in both the sexes is similar irrespective of the character brought by the male or female parent. These results convinced Morgan that the inheritance of the character of eye colour was in such a way that the gene determining it was located on the X-chromosome and red eye was dominant to white eye. The results of such prosses are shown in Fig. 21.2A, B, and C.

The above results are in agreement with the hypothesis that the gene is carried by the X-chromosome and not by the Y-chromosome. Inheritance of characters in this fashion is called sex linkage and the related characters are termed sex-linked. Since the female possesses two X-chromosomes, she may be either homozygous or heterozygous for an X-linked gene, whereas the male possesses only one representative of such a gene, and so he is termed hemizygous.

(6) Fig. 21.2 A. B & C Sex linkage in red eye character in Drosophila.

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#### Sex Linkage in Man

Colour blindness is a sex-linked character. The gene carrying it is recessive. Suppose a woman heterozygous for colour blindness marries a normal man. Among the offspring, all daughters would be normal, while half the sons would be colour-blind and half normal. Among the daughters, half would be heterozygous for colour-blindness and hence they would be potential carriers of this character. So if one of them marries a normal man, half of the male offspring would be colour-blind. The results are indicated in Fig. 21.3.

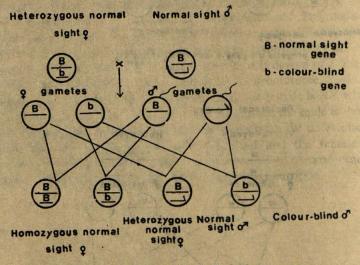


Fig. 21.3 Sex-linked character of colour-blindness in man.

The above study of sex linkage points out that if a lethal or harmful gene is linked, then half the males are likely to be affected. This is because, half of the male progeny would receive the gene from their heterozygous mother.

#### Heredity through Y-Chromosome

In Drosophila, the sex-linked genes are present only in the X-chromosome while their alleles are absent in the Y-chromosome. Hence, the recessive character carried by the gene in the male appears in the phenotype. So it is concluded that the Y-chromosome is 'empty' or 'inert' at least in comparison with the X-chromosome. There are, however, exceptions to this. For example, one gene blobbed has alleles both in the X- and Y-chromosomes. When the recessive mutant allele of the blobbed gene occurs in both the X-chromosomes of a female, the bristles present on the body of the fly become shorter and more slender than the normal ones. The bristles of the male in whom the blobbed gene is present in the X-chromosome and its normal allele in the Y-chromosome, are normal. If this male is crossed with a blobbed female, all the male progeny would be with normal bristles and all the females with blobbed bristles, because the character of normal bristles is inherited through the Y-chromosome of the male parent. Several blobbed alleles have been found in the Y-chromosome. Males carrying blobbed gene both in the X- and Y-chromosomes possess blobbed bristles.

It is claimed that in man several characters carried by linked genes in the Y-chromosome are inherited. According to Stern (1957), however, these claims are doubtful. Dronumaraju (1965) stated that the condition of hypertrochosis is because of Y-chromosome linkage, while Mittwoch (1967) opines that this is debatable.

# Non-disjunction of Sex Chromosomes and Proof of **Chremosomal Basis of Heredity**

Sex-linked genes are often used by geneticists to get information regarding sex determination. Bridges (1913-16) performed a series of experiments which proved to be highly successful in obtaining crucial evidence for the gene-chromosome hypothesis. While carrying out breeding experiments, he obtained a strain carrying the sex-linked gene vermilion eye. If a female with a vermilion eye is crossed with a normal red-eyed male, it would be quite reasonable to expect  $F_1$  progeny consisting of red-eyed females and white-eyed males. Bridges also observed the presence of a few white-eyed female and red-eyed male flies. This was unexpected. He crossed these exceptional white-eyed females to normal red-eyed males. The progeny were mostly white-eyed females. Cytologically he found that they possessed not only two X-chromosomes but also a Y-chromosome. In meiosis, the XXY female flies could form four types of gametes in regard to the sex chromosomes, namely, XX, Y, X, and XY. Actually, the gametes were XY or X, in over 90% cases; they were XX or Y in less than 10%. The results are illustrated in Fig. 21.4.

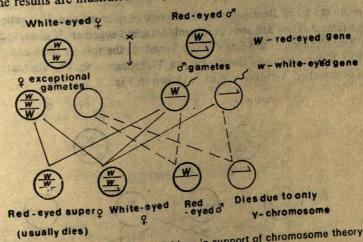


Fig. 21.4 Proof provided by Bridges in support of chromosome theory.

In case of the above mentioned crosses, Bridges suspected that meiosis in the female parent failed with the result that both the X-chromosomes went to the same pole and thus were included in the same gamete. The failure of meiosis thus, led to the formation of some eggs with three X-chromosomes while others with none. Such a failure of chromosome separation is called non-disjunction. The progeny with no X-chromosome die and those with three X-chromosomes usually do not survive up to the adult stage. Hence, in the case of non-disjunction where two X-chromosomes pass to one pole, the offspring are like their parents, i.e. white-eyed females and red-eyed males (Fig. 21.4).

When Bridges examined the cells of a white-eyed female, he found as per expectations two X-chromosomes and a Y-chromosome. In case of a red-eyed male, the cells showed only one X-chromosome and Y-chromosome was absent. These observations made him lead to the conclusion that whenever there was an exception in the inheritance of the eye colour, there was corresponding exception in the chromosome constitution. This was the convincing proof in favour of the chromosomal basis of heredity. It showed that genes are situated in the chromosome and completely removed any doubt, whatsoever, about the correctness of the chromosome theory of heredity.

The above information was very helpful in getting a deeper insight into the basis of sex determination in Drosophila. It indicates that Y-chromosomes have nothing to do with sex determination. There can exist a female with a Y-chromosome and a male without it. So it may be concluded that the sex is determined by the number of X-chromosomes and not by the Y-chromosomes. In Drosophila, however, the presence of Y-chromosomes is necessary for the normal development of sperms. XO-male flies are sterile.

Non-disjunction is not limited to the sex chromosomes. It may occur with any of the chromosomes. A Drosophila fly possessing an extra autosome or a missing one usually dies. There are, however, exceptions. Haplo-IV possessing a single chromosome IV and Triple-III with three chromosomes IV are viable, but show characteristic abnormalities.

could form four types of gameros in regard to the sex chromosomes, namely, XX, Y, X,

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There are some stocks of Drosophila where there is permanent non-disjunction of X-chromosomes in the female. They are seen as if firmly attached together. If observed under a microscope, two X-chromosomes are found to be joined at the spindle fibre end. The female flies with attached X-chromosomes usually carry a Y-chromosome. The characteristic pattern of inheritance of attached X-chromosomes is shown in Fig. 21.5.

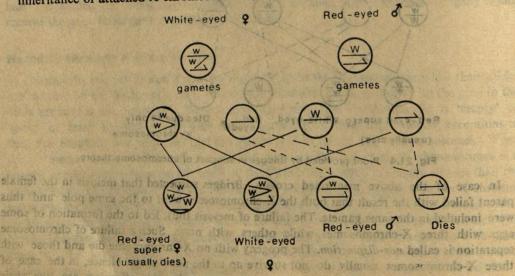


Fig. 21.5 Inheritance of attached X-chromosomes in Drosophila. parents i.e. white-eved females and red-oved males

The zygote forms four types of gametes. Of them, the one with three X-chromosomes and the one without X-chromosome are usually non-viable. Therefore, two types which survive are similar to their parents. This is in contrast to the normal pattern of sex-linked inheritance in which the X-chromosome of the male is from the mother.

- 1. In 1902, McClung showed that the somatic cells in male and female grasshopper contained different chromosome members and the specific chromosomes formed an unequal pair in the other sex. These are called sex chromosomes and the rest autosomes. In man or Drosophila, the male possesses an unequal pair of sex chromosomes, one called the X- and the other the Y-chromosome, while the female has an equal pair, so both the members of the pair are X-chromosomes.
- 2. The discovery of sex linkage by Morgan in 1910 is a very important event in the history of chromosome theory. The case of inheritance of red and white eyes in Drosophila indicates that the gene for eye colour is carried by the X-chromosome. Inheritance of characters in this fashion is called sex linkage and the related characters sexlinked. Colour blindness in man is also a sex-linked character.
- 3. In Drosophila, the sex-linked genes are present only in the X-chromosome while the Y-chromosome is inert. It is claimed that in man several characters carried by linked genes in the Y-chromosome are inherited but Mittwoch opines that this is debatable.

Bridges observed formation of eggs with 2X-chromosomes in Drosophila, and others with none due to the failure of separation of X-chromosomes in meiosis. This failure is called non-disjunction. This example is convincing proof of the chromosomal

4. There are some stocks of Drosophila where there is permanent non-disjunction of X-chromosomes in the female.

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# 22. Sex Determination and Differentiation

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The fundamental characteristic of sexual reproduction is the union of two different gametes. In all higher animals, sexual union is the only or principal method of reproduction. In plants and also in many lower organisms, this is the usual method of reproduction.

In some lower organisms, the gametes which unite outwardly appear similar to each other but functionally they may be different. In all other organisms, male and female gametes are distinctly different from one another. The egg is much larger than the sperm and less active. Because of such a basic difference between male and female individuals, sex is differentiated possessing more or less distinct different features.

Now the question is whether the sex can be controlled. Many attempts have been made uptil now to have the desirable sex of an embryo developing within the uterus of a woman. Dieting experiments have been made on the mother before the birth of a baby with the object of finding out whether this has the desirable effect on the sex of the embryo. However, all such attempts have been unsuccessful. One of the reasons for the failure being that sex of the embryo is determined at the time of fertilisation of the egg. It means that whether the zygote will develop into a male or female individual is decided at this time (Fig. 22.1).

There are various theories about sex determination. According to Hicker (1920), these can be classified into three groups: (i) sex determination before fertilisation of the egg—progamic, (ii) sex determination at the time of fertilisation—syngamic, (iii) sex determination after fertilisation during the developmental stage of the embryo at any time—epigamic. The modern theory of sex determination is based on the progamic or syngamic idea.

We should bear in mind that sex determination is not the same as sex differentiation. Sex differentiation starts only after fertilisation of the egg. The zygote which is at first undifferentiated, undergoes differentiation during the development of the embryo. This results in the differentiation of the body in such a way that the sex can be differentiated. Currently, there are three ways of finding out the sex of an unborn child: (i) Examination of cells in the vaginal tract. The pycnotic body containing chromatin and which is attached to the nuclear membrane becomes deeply stained. After that, it is carefully observed. (ii) Amniocentesis, i.e. examination of the amniotic fluid around the embryo. This fluid is collected and examined for the heteropycnotic body. (iii) Biochemical examination of the blood of the pregnant woman.

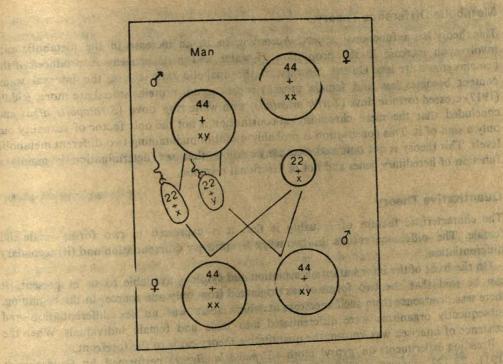


Fig. 22.1 Sex determination in man.

## Theories of Sex Determination

There are mainly five theories proposed to explain sex determination: (i) Alternate dominance (Castle), (ii) Metabolic difference theory (Riddle), (iii) Quantitative theory (Goldschmidt), (iv) Theory of heterozygosity (Correns) and (v) Gene balance theory (Bridges). These theories are based on two assumptions: (i) Sexuality is hereditary and it is controlled either by Mendelian genes or specific chromosomes. Its expression may be complex. However, it is not, different from the expression of other characters (Bateson, Emerson, Correns and others). (ii) Sexuality is functional and dependent on environment (Riddle, Goldschmidt a very summarie and the track principle principle of the contract of the contr and others.)

Theory of Alternate Dominance Castle (1903) proposed provisionally a hypotheiss on sex differentiation. His theory of alternate dominance is based on three assumptions: (i) All reproductive cells are heterozygous from the point of sex; (ii) the gametes of only opposite sexes unite; (iii) sex character follows the law of alternate dominance. In the male progeny, male determinants are dominant while in the female progeny, female determinants are dominant. This theory has, however, been rejected because it does not explain the cause of alternate dominance.

#### **Metabolic Difference Theory**

This theory has a functional aspect. According to it, an increase in the metabolic rate involves an increase in the proportion of water and male progeny is produced if the proteins stored are less. On the contrary, if the anabolic rate is less, the internal water content becomes less and female progeny is produced if proteins stored are more. Riddle (1917) crossed tortoise dove (*Turtur orientalis*) with white ring dove (*Stratopolis alba*) and concluded that the mere chromosomal constitution is not the only factor of sexuality but only a sign of it. This constitution is probably useful in maintaining two different metabolic levels. This theory is not outdated, because we know that sex determination is mainly a function of hereditary genes and not of functional response.

#### **Quantitative Theory**

The characteristic feature of sexuality is that it is expressed in two forms—male and female. The difference results in two ways: (i) primary differentiation and (ii) secondary differentiation.

On the basis of the information on evolution and cytology available to us at present, it can be said that the two forms of sex originated from only one source. In the beginning, there was divergence from such ancestors in whom there was no sex differentiation and subsequently organisms were differentiated into male and female individuals. When the existence of inter-sex was known, the quantitative theory came to the forefront.

Breeding experiments on 'gypsy' moth (Liemandria dispar) performed by Goldschmidt (1911, 23, 27) showed gradation of inter-sex between the normal male and female. While explaining this abnormality, he suggested that an enzyme called andrase (theoretical) might be leading to maleness and an enzyme called gynase (theoretical) to femaleness. He also stated that the inter-sex at first starts either as a male or female and develops as such for some time but subsequently becomes an inter-sex.

### Theory of Heterozygosity

Correns proposed this theory in 1906. According to it, only one parent is heterozygous and the other is homozygous. It has received a lot of support today.

#### Sex Chromosome

We have already seen that the credit for the discovery of sex chromosome goes to Henking (1891). He observed a specific chromatin object in *Pkyrhocoris apteris*. It was in a condensed form in the beginning of prophase of the primary spermatocyte. He described it as a false nucleolus. Paulmier (1898) observed a similar phenomenon in *Anasa tristis* and de Sinety (1901) in *Orphania*. McClung found an odd chromosome always without a partner. He suggested that it was concerned in the sex determination in grasshopper. Soon after, Stevens (1905, 1906, 1908, 1909), Wilson (1905a, b, 1906, 1909) and other workers observed the same chromosome without a partner in the male and two 'odd' chromosomes in the female grasshoppers. This odd chromosome was called the X-chromosome or sex chromo-

some because one of them was characteristic of the male and two or the female. Sex chromosomes have also been given several names, for example, 'special chromosome' (Sinety); 'odd chromosome' (McClung); 'heterotropic chromosome' (Wilson); 'monosome' (Montgomery); 'chromosome X' (Montgomery); 'heterochromosome' (Montgomery) and 'allosome' (Montgomery). The term which is often used to indicate the sex chromosome is 'allosome'. in distinction to that applied to all the other chromosomes which are termed 'autosomes'.

## Mechanism of Chromosome Sex Determination

#### Male Heterogamety

#### Protenor Type

This type was first described by Wilson in 1905 in the bug Protenor halfrager. As there is an equal number of male-producing sperms, the sex of the future individual depends on the chance of which type of sperm, whether simplex or duplex for the X-chromosome, fertilises the egg. The chromosomal formula, if n represents the haploid number of autosomes in a given species, is 2n + XO for the male and 2n + XX for the female.

## Lygaeus and Drosophila Type

The X-chromosome of the male in this type has a partner called the Y-chromosome. However, X and Y are non-homologous or only partly so. The female possesses a pair of X-chromosomes which are homologous throughout their length. The shape and structure of the Y-chromosome are different from those of the X-chromosome. Two types of male gametes are produced and thus the digamety of the male sex is achieved through this mechanism. The Y-chromosome is transmitted by the male parent. This type was first described by Wilson in 1906 in Lygaeus turcius. The chromosomal formula for the male is 2n+XY and 2n+XX for the female. Such is the case in majority of plants and animals the purpose of the bounded of the state of the state of including man (Fig. 22.2).

### **Female Heterogamety**

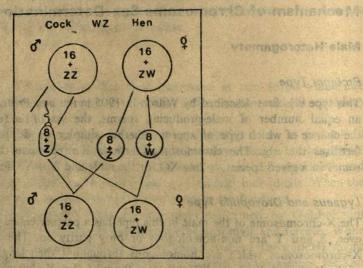
In this type, the female is digamatic. This has been analysed cytologically in Lepidoptera (Seiler, 1914a, b, 1920). The chromosomal formula is 2A+ZZ for the male and 2A+ZW for the female. Similar is the case with poultry (Fig. 22.3). Besides, XX/XI, XX/XB and ZZ/ZW types rarely occur.

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## Cytology of Sex Chromosome

Sex chromatin was first observed by Barr and Bertram (1949). During the growth period of spermatocytes of many animals, the X-chromosome becomes highly condensed and often STRONG TO BE

assumes a round shape. It stains deeply with basic dyes and shows the sharpest contrast to the highly staining autosomes. These X-chromosomes are J-shaped in Hipponae, V-shaped in Texopneustes and rod-shaped in Euchenops. During meiosis, X-chromosomes divide equationally in one division while in the second division or heterokinesis they may involve either preheterokinesis (in first division) or post-heterokinesis (in second division) of the spermatocyte (Guthers, 1907). The interesting feature of heterokinesis is that the disjunction of X-chromosomes precedes the other in some cases (precession) while in other cases, it remains behind them (succession).



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Fig. 22.3 Sex determination in poultry.

According to Schrader (1920, 1923), there is some reason to suspect that the sex chromatin may have primarily been widely distributed in all the sex chromosomes. A similar condition of this kind may have persisted in many cases with little or no modification. Perhaps it may be because of this that there was failure to observe sex chromosomes in many species. It is likely that chromatin might have become localised in one of the chromosome pairs which were originally homozygous (XX) but later became heterozygous (XY). This origin of the Y-chromosome may be on account of erosion of some parts of the X-chromosome.

In some dioecious plants, there are X- and Y-sex chromosomes, as in animals. Pistillate plants possess two X-chromosomes while staminate plants have X- and Y-chromosomes. These two types are usually referred to as females and males. Botanically, however, this terminology is not correct, because all plants are asexual bearing spores. Pistillate plants form megaspores that give rise to the gametophytic generation which results in the formation of female gamete and egg nucleus. Similarly, staminate plants form microspores (pollen grains), which give rise to the gametophytic generation producing male gametes. The pistillate plants are generally referred to as 'females' and staminate ones as 'males'. Thus, you will see that the end result is the same, i.e. the pistillate plants forming eggs and staminate plants sperms. Functionally, pistillate and staminate plants are females and males respectively, and so designated by several authors.

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## Multiple Heterochromosomes

There are examples where X- and Y-chromosomes, or both, are present in multiples. In Ascaris megalocephala, (XO/XX), 9X components have been observed.

## Genic Balance Theory of Sex

Bridges (1925) proposed this theory and put forward evidence in its support. According to him, both autosomes and allosomes have a part in sex determination. The sex chromosomes carry the female tendency and the autosomes the male tendency. The latter is ZA+XY since the female tendency of the single is not strong enough to overcome the male tendency of the combination of genes situated in ZA chromosomes. Similarly, 2A+XX is a female since the double dose of the female tendency residing in 2X chromosomes is sufficiently strong to overcome the male tendency of the autosomes.

Polyploidy is prevalent in Drosophila. Bridges took advantage of this and obtained a series of flies that showed certain cytological compositions in respect of X-chromosomes and autosomes, as indicated in the Table 22.1.

Table 22.1 Sexual types in Drosophila melanogaster (after Bridges)

| Sex          | X-chromosome | Sets of autosomes  | Sex index ratio  |
|--------------|--------------|--|--|
| 一            |              | 7 3 - 2 - 2 - 2 - 2  | 1.5  |
| Super-female | 3            | THE WAY  | 1.0  |
| Tetraploid   | 4 4          | 26.24  | 1.0  |
| Triploid     | 3            | 2  | 1.0  |
| Diploid      | 2            | 1  | 1.0  |
| Monoploid    | 1            | 5  | 0.67   |
| nter-sex     | 0 2 2        | 2 6  | 0.50   |
| Normal male  | 7 1          | 3  | 0.33   |
| Super-male   | 1            | A SECTION OF THE SECT | THE PARTY OF THE P |

# Single and Multiple Gene Sex Determination

There are apparently a number of genes on the X-chromosome of Drosophila that cumulatively cause its female producing tendency. On the other hand, in mosquito, the sex determining potency of X- and Y-chromosomes is due to a single pair of genes. The Ychromosome is very similar to the X-chromosome in appearance in mosquito and homologous to it (X). It carries alleles of the genes of the X-chromosome. Individuals homologous for a gene m are females while Mm individuals are males. Hence, here it is appropriate to refer to them as sex genes instead of sex chromosomes. lacrosca en bua sau tollas canto merclares de eléctro-ello hactogras con en a

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## Sex Determination in Honey Bee

In honey bee (Apis mellifera) (Fig. 22.4), males are formed from unfertilised eggs through a particular kind of parthenogenesis termed 'arrhenotolly' or haploid parthenogenesis. The female bees are, however, born from fertilised eggs. The sperms produced by the male are stored in the queen bee in a seminal receptacle within her body and are always available for fertilising eggs produced throughout the remainder of her life. When she lays an egg in a worker cell, sperms are ejected from the seminal receptacle for fertilising the egg, which after fertilisation will develop into a female bee. When the queen comes to a drone cell, she exerts some kind of pressure on the duct leading from the seminal receptacle so that the sperms are unable to pass out and fertilise the egg as the latter passes down the oviduct. So an unfertilised egg will be laid which will subsequently develop into a male.

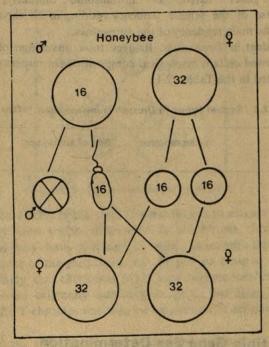


Fig. 22.4 Sex determination in honeybee.

## Non-Genetic Control of Sex Determination

There are a few animals where the sex is determined by environment. In the worm Bonell, the larva is free-swimming. If it settles to the bottom of the sea without having any conta with any other of its kind, it develops into a female. But if it touches a female, it develop into a male and subsequently passes into its uterus to lead a parasitic life. In this case, the embryo is potentially capable of developing into either sex and an external stimulus trigger the development one way or the other.

#### Sex Determination in Plants

In most of the Bryophytes, the haploid gametophytes exist into two forms, female (XA) and male (YA). Allen (1917) described the large X in the female gametophyte and the small Y in the male gametophyte in Sphaerocarpus donnellii. When the sperm unites with the egg, a diploid zygote is formed possessing 14 autosomes and one X- and one Y-chromosome. It develops into a diploid sporophyte which forms haploid spores through meiosis. These spores carry only one of the sex chromosomes. They mature into male and female gametophytes depending upon which of the two chromosomes they receive.

According to Allen, who reviewed the position in 1940, there are about 40-45 Bryophytes possessing identifiable sex chromomes while in about the same number of species studied,

there are no distinguishable chromosomes.

As regards higher plants, Blankburn (1923) and Winge (1923) first described sex chromosomes in Melandrium (Lychnis), Humulus and other species, in Rumex by Kihara and Ono, and in Elodea by Santos.

Allen summarised the data on sex determination in 117 species of angiosperms in 1940. Of these, X- and Y-chromosomes were shown in about 55 species while in 16 species a definite type of mechanism different from the X-Y mechanism was demonstrated. No sex determining mechanism could be, however, recognised in 46 species.

Lindsay (1930) classified the known cases of sex determination into the following:

1. XY type: In this case the male is digametic and heterogamous for sex while the female is monogametic and homozygous. The chromosome formula for the male is 2A + XY and 2A+XX for the female. Elodea coccinia is an example of this type.

2. This is similar to (1) but includes tri- and tetrapartite groups.

Tripartite: The male is digametic and heterozygous for sex. The chromosome formula is 2A+Y<sub>1</sub>Y<sub>2</sub> (male) and 2A+XX (female). In this type, Y<sub>1</sub> and Y<sub>2</sub> pass to one pole and X to another pole during meiosis.

Tetrapartite: In this type, X and Y are in the duplex state in males and 4X in females. The chromosome formula is  $2A + X_1X_2Y_1Y_2$  (male) and  $2A + X_1X_2X_1X_2$  (female). This type is found in Humulus lupulus. In case of males, X1 and X2 go to one pole and Y1 and Y2 to

the other. Such a type of sex chromosome complex does not exist in animals.

3. XO type: In this case, the male is digametic and the female monogametic and homozygous. The chromosome formula for the male is 2A + XO and 2A + XX for the female. This is observed in Dioscorea sinuata (Meurman, 1925). However, recently Ramchandran (1962) has demonstrated that XY type exists in the male plants of tetraploid D. tomentosa and D. pentaphylla, octaploid D. bulbifera and in the nineploid D. spinosa.

4. Special type: In Fragaria eletior, the male is monogametic and homozygous while the female is digametic and heterozygous. The chromosome formula for the male is 2A + ZZ

and 2A + WZ for the female.

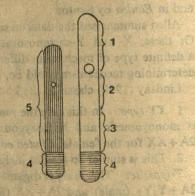
Westergaard (1958) published an exhaustive review of the work regarding sex determination in the known cases. Melandrium (Lychnis) is a plant in which sex determination has been worked out in great detail (Ono, 1939, 1940; D'Love, 1940, 1942, 1944; Westergaard, 1940, 1953; Warmke, 1946). In this plant, the sex is determined by a pair of XY chromosomes as in many animals. There are 12 pairs of chromosomes in the normal diploid. Of them, 11 pairs are of autosomes and there are X- and Y-chromosomes. The X- and Y-chromosomes are vastly different morphologically (Fig. 22.5). Pistillate or female plants are XX and staminate ones are XY. The former is determined by the absence of the Y-chromosome rather than by the presence of two X-chromosomes.

Warmke induced polyploidy in the normal diploid plants of *Melandrium* by using colchicine. He observed that the Y-chromosome is the most important in sex determination. According to him, one Y-chromosome is equal to approximately four X-chromosomes in determining sex. In this plant, the balance is between the X and Y, and the number of auto-

somes has no effect in contrast to the condition in Drosophila.

The Y-chromosome in *Melandrium* is physically larger and more conspicuous than the X-chromosome. It carries the factors for maleness. Analysis of the effects of fragments of the Y-chromosome on the expression of sex has helped research workers to designate some degree of localisation of functions to different portions of the Y-chromosome. This is shown in Fig. 22.5.

Fig. 22.5 Sex chromosomes in Melandrium (diagrammatic). Regions 1, 2 and 3 of Y-chromosome do not have homologous parts in the X-chromosome and hence, they form the differential portion of the Y-chromosome. Regions 4 are homologous in the X and Y and so they behave as pairing regions in meiosis. Region 5 is the differential part of X. When 1 is lost from the Y, a bisexual plant is obtained. If 2 is lost, a female plant is produced. If 3 is absent, male sterile plants with abortive anthers are obtained (after Westergaard, 1958.)



Recently, Roy (R.P.) and Roy (P.N.) (1971), and Roy (R.P.) and his coworkers (1972) have reported an interesting case of sex determination in *Coccinia indica* of the Cucurbitaceae. In this plant, a dimorphic pair of chromosomes is clearly observed (Fig. 22.6). Roy (R.P.) et al. tested the heterogamety of the males and determined the specific role of the complements of the heteromorphic pair in the expression of sex. They adopted the same procedure of inducing polyploidy by colchcine and crossing polyploids with diploids to score the sex of the progeny as was done in the case of *Melandrium*.

Colchicine-induced tetraploid of *Coccinia indica* when crossed to a diploid male produced 16 females and 14 male plants (i.e. 1:1 ratio), proving that the male is heterogametic and not the female. When examined cytologically, the tetraploid female plant had almost all the chromosomes alike (Fig. 22.6A), whereas in the tetraploid male, a single Y-chromosome

(Fig. 22.6B) was conspicuous by its long size. Roy (R.P.) et al. concluded that:

- (i) there is an XX and XY sex mechanism in Coccinia indica,
- (ii) male is the heterogametic sex, and
- (iii) Y-chromosome plays a significant role in sex determination.

A plant with all 24 chromosomes alike and having a chromosome constitution XX + 2A is a female at the diploid level in *Coccinia indica*. But another plant with 24 chromosomes

having a big Y-chromosome in the somatic plate and showing a heteromorphic pair at meiosis is a male with a chromosome constitution XY+2A. A trisomic (25 chromosomes) with two Y-chromosomes (Fig. 22.6C) in the somatic metaphase and a trivalent with one X- and two Y-chromosomes at meiosis is a male plant with a cluster of male flowers (Fig. 22.6D). In a triploid with 36 chromosomes where Y is lacking, the plant is a female. At the tetraploid level, there are two plants with 48 chromosomes, one in which all chromosomes are alike at somatic metaphase (Fig. 22.6A). Evidently, it has no long Y-chromosome and the plant is a female. But the other plant with 48 chromosomes has a solitary long Y-chromosome at somatic metaphase (Fig. 22.6B). It forms a quadrivalent at meiosis in which a single long Y-chromosome is associated with three small X-chromosomes forming a chain and the plant is male. In this plant, the role of Y-chromosome is clearly established because the single Y is able to neutralise the effect of three Xs.

In Salix species, there is no duplication of the XY pair in the tetraploid and hexaploid while the autosomes only duplicate. In Empetrum, in case of the tetraploid species, however, the XY pair also undergoes duplication.

An analysis of the sex determining mechanism in diploid and polyploid plants clearly indicates the existence of two types of trigger mechanisms. One type possesses an active Y-chromosome which plays a decisive role in sex determination. In the other type, the X-chromosome/autosome ratio is a decisive factor while the Y-chromosome is inactive (Humulus).

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In Jackfruit (Artocarpus integrifolia), cases have been recorded where the male inflorescence has become carpellate (Venkațarayan, 1949; Sahadevan et al., 1950). În coconut (Cocos nucifera), Narayana and John noted a complete male plant. In Carica papaya, there are examples of a male tree becoming completely carpellate after the crown was cut down. There are reports that environment also plays an important part in causing sex reversals and intersexes in nature. For example, Schafner (1931) reported that soil conditions could also a to about the about the man about the sales and the change the sex in Cannabis sativa. of some and the art of the state of the second design of the state of

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#### **Autonomous Type**

In Drosophila, each cell develops into a male or female tissue depending on the chromosome constitution of that specific cell. Such a development is called autonomous. Any character for which the expression depends only on the cells that make up the structure concerned and is not influenced by other parts of the individual is said to develop autonomously. An excellent example of this type is what is called sex 'mosaic' or 'gynandromorph' in Drosophila (Fig. 22.7). This has been found to be fairly frequent as an abnormality in Drosophila stocks. In these flies, which at first start out as normal females, one of the X-chromosomes gets lost at an early stage of cleavage with the result that the descendants of that specific cell are all male instead of female. Hence, the adult fly is patly male and partly female. The line of demarcation between the two types of tissues is also very distinct.

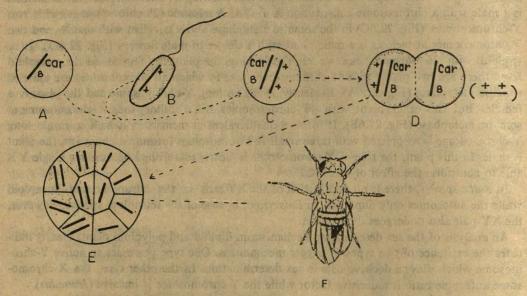


Fig. 22.7 The origin of gynan dromorph in *Drosophila* and some of its possible effects. The sperm carrying X-chromosome (B) unites with the egg (A) carrying the gene Barreye and sex-linked gene carnation (car) and the zygote (C) is produced. But at its first division, one X-chromosome is lost (D). Hence, in the developing embryo, half the part contains 2X-chromosomes in the cells. While in the other half, cells are without one X-chromosome. So the former is like the female and the latter like male. Hence, F is the gynandromorph,

### Non-autonomous or Hormonal Type

In vertebrates, the embryo is capable of developing into either sex. In them, the chromosome make up seems to start the developmental sequence along one or the other of these paths. Further development is mostly under the control of hormones. The process may be reversed at any stage because of a strong environmental influence and it may then start along the other path. The following example will serve as an illustration.

#### 1. Free Martin

In case of twin calves where one is a male and the other a female, the latter generally develops into a sterile intersex-like animal called a *free martin*. It is because in cattle, there is an opportunity for the blood of twins to mix because of occasional inter-connections between their blood vessels. Therefore, it seems that the male calf produces a hormone which depresses the sexual development of his twin sister.

## 2. Sex Reversal in Man, Chicken and Amphibians

There are several instances of a woman changing her sex and becoming a man or vice-versa during the life time of an individual.

In birds, the ovary develops on the left, whereas the gonad which is on the right remains undeveloped. Sometimes however hens change into cocks (males) in later life. In most of these instances, the ovary had become diseased or had got destroyed. Because of this, the right gonad developed into a testis and produced sufficient hormone so as to change the sex of the hen. In the amphibian Amblystoma, the females were altered into males by temporarily grafting a testis onto the developing larva.

#### SUMMARY

- 1. In unisexual or dioecious organisms, an individual will produce usually only sperms or eggs. Because of such a basic difference between male and female individuals, sex is differentiated, possessing more or less distinct different features.
- 2. The sex is determined at the time of fertilisation of the egg. There are various theories about sex determination. As regards the mechanism of sex determination, male heterogamety is found in *Protenor*, *Lygaeus* and *Drosophila* types and female heterogamety in the *Abraxas* type. In some dioecious plants, there are X- and Y-sex chromosomes. Pistillate plants possess two X-chromosomes while staminate plants have X- and Y-chromosomes.
- 3. In 1925, Bridges proposed the genic balance theory of sex. According to it, both the autosomes and sex chromosomes have a part in sex determination. The sex chromosomes carry the female tendency and the autosomes the male tendency.
- 4. In *Drosophila*, there is multiple gene sex determination while in mosquito, the sex determining potency of X- and Y-chromosomes is due to a single pair of genes. There are a few animals where sex is determined by environment, e.g. *Bonellia*.
- 5. There are several Brophytes possessing identifiable sex chromosomes. Allen summarised the data on sex determination in 117 species of Angiosperms in 1940. Of these, X- and Y-chromosomes were shown in about 55 species. The XO type is observed in Dioscorea sinuata, while there are special types of sex determination in plants like Fragaria eletior, Melandrium (Lydhnis) and Coccinia indica.
- 6. Sex differentiation takes place after fertilisation of the egg. There are two types: autonomous type and non-autonomous or hormonal type. *Drosophila* is an example of the former while vertebrates come under the latter type. As there is an opportunity, in cattle, for a mixture of the blood of twins because of occasional interconnections between their blood vessels, a free martin may develop. There are several instances of sex reversal. These are of the hormonal type.

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## 23. Chromosomal Aberrations

Chromosomes undergo various types of changes which may affect the inheritance of one or more characters. Most higher plants and animals contain two sets of chromosomes, one derived from the male parent and the other from the female parent. Such organisms are, therefore, diploid. Their gametes will have only one set of chromosomes, thus making them haploid or monoploid.

Chromosomal changes are of two types: (i) changes within the individual chromosomes called *chromosomal aberrations*, and (ii) changes in the total number of chromosomes which usually result in the organisms having more number of chromosomes than their diploid

counterpart. This process is known as polyploidy.

We have gained much knowledge in respect of chromosomal aberrations from the extensive researches on the salivary gland chromosomes of *Drosophila* and pachytene chromosomes of maize. Although chromosomes possess a definite organisation, they can undergo changes. If they are broken, their normal structure gets disrupted. Some of the agents which cause breaks in chromosomes are radiation (e.g. x-rays, gamma rays) and various chemicals. Chromosomal breakages may occur even under natural conditions, but in most cases, it is not possible to know the nature of the breakage.

# Breakage-Fusion-Bridge Cycle

McClintock (1941) has done extensive work on broken chromosomes in maize. She found that in the gametophyte and endosperm of the plant, ends of chromosomes which had recently been broken were behaving as though they were 'sticky'. In other words, broken ends have a tendency to adhere to one another. When a broken chromosome undergoes duplication during cell division, the two sister chromatids may adhere at the point of previous breakage. McClintock has observed that when such a situation takes place, certain consequences arise. These are shown in Fig. 23.1.

The fused sister chromatids are not able to separate readily. So a single chromatid with two centromeres (kinetokores) is formed. It is known as a dicentric chromatid. When the centromeres move to opposite poles at anaphase, the dicentric chromatid becomes stretched out with the result that a chromatin bridge is formed extending from one pole to the other. Because of tension, this bridge ultimately breaks. The breakage, however, does not always occur at the point of the previous fusion. Hence, chromosomes may be formed having duplication or deficiency as compared with the original chromosome. For example, if the original chromosome is of the G F E type, the type G F E E will be a duplication because of the

occurrence of the region E twice while the type G E will be a deficiency as the region is deficient in E.

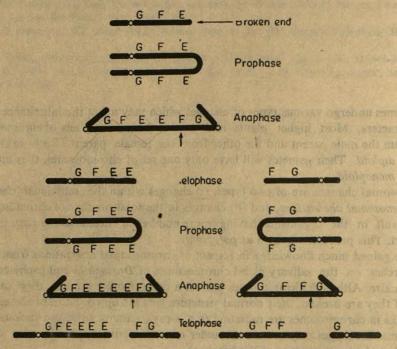


Fig. 23.1 Breakage-fusion-bridge cycle.

The above situation can cause repetition in cyclic series of more events similar to those diagrammed above. Such a cycle is known as a breakage-fusion-bridge cycle.

Chromosome aberrations can occur spontaneously through the breakage-fusion-bridge cycle for some time. However, when a broken chromosome is introduced into the sporophytic generation, the cycle ends, because of healing of broken ends in the zygote.

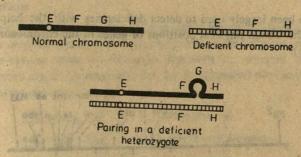
Chromosomal aberrations are of four types: (i) Deficiencies or deletions, (ii) duplications, (iii) inversions, and (iv) translocations.

#### **Deficiencies**

The simplest type of chromosomal aberrations is a deficiency or deletion. In this case, the chromosome is devoid of a short segment. Usually, the missing segment is an internal one. The end of the chromosome is usually not involved in a deletion. Figure 23.2 shows a deficiency in one chromosome in the homozygous condition.

Figure 23.2 indicates that when a deficiency occurs in one of the homologous chromosomes, the unpaired portion of the unaffected homologue forms a loop or bulge while pairing. If any genes were present in the missing segment, their alleles would be expressed in the hemizygous condition.





Deficiency. In the deficiency heterozygote, region G of the normal chromosome bulges out since this part has no corresponding region in the deficient chromosome.

Suppose we know that the gene A is dominant over a and produces a certain phenotype. Suddenly, we observe that in a heterozygote the recessive allele is expressed. Under such a condition, we should expect that probably a deficiency has occurred and the segment carrying the dominant allele A was deleted and so the recessive allele got a chance to express itself (pseudodominance). So this is one of the methods of positively identifying a linkage group with a known chromosome. In maize, if the silks of a recessive phenotype will appear then we should suspect a deficiency involving a chromosomal segment carrying the dominant alleles. If the recessive type is carefully examined, it may be possible to pinpoint which of the ten chromosomes has a deficiency.

Geneticists have used deficiencies to find out what happens when certain genes are absent and what they do when present. For instance, in Drosophila, when a segment of the chromosome containing the locus of a certain gene is missing, the eye becomes white instead of red. So we infer that the missing segment is concerned with the formation of red pigment. It has been observed that when certain segment of the X-chromosome in Drosophila is missing, the wing possesses a notch in the outer borae. All these cases have helped in understanding better the part played by the normal genes in this region. Large deficiencies are mostly lethal since they are likely to contain vital genes.

Deficiencies have been used to locate the actual physical position of genes in chromosomes and to prepare their maps. Such maps are called cytological maps.

### **Deficiencies in Cytological Mapping**

If a heterozygous individual shows pseudodominance as a result of a deficiency, causing the expression of recessive genes, the deficiency can be visually identified. Geneticists therefore have a tool for detecting loci involved in a deletion as well as for the cytological delimitation of deletions of a good size. If certain genes are shown to be absent in the case of a specific deletion and a specific segment is also observed to be missing, then it can be reasonably inferred that the genes in question are located in the missing segment. While preparing cytological maps, it is necessary to study short deficiencies involving a few cross-bands as well as identify as many gene loci as possible. Another method is to use a series of more extended deficiencies which overlap one another in varying degrees. Salivary gland chromosomes

in *Drosophila* have been largely used to detect deficiencies with the help of the cytological maps indicating the actual physical positions of genes in the chromosomes that have been prepared (Fig. 23.3).

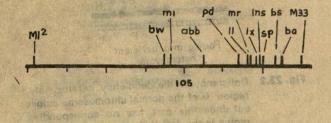




Fig. 23.3 Cytological map (lower) of the part of end of right limb of chromosome II of *Drosophila mel—anogaster*. For comparison, the corresponding map based on linkage (upper) is also shown.

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### **Duplications**

We have already seen how deficiencies or deletions occur. The same mechanism may be responsible for duplication of chromosome segments. Frequently, the duplication of even a small chromosomal segment may cause modification of the phenotype.

Even before the discovery of salivary gland chromosomes in *Drosophila*, Sturtevant and Morgan (1923) studied extensively the Bar mutant in *Drosophila*. They found that this mutant was not an ordinary one. It was really a case of duplication of a small fragment of the X-chromosome. They observed that Bar did not always breed true. In the previous chapter, we saw that there was a small percentage of cases where normal flies as well as extreme bar flies were produced as a consequence of crossing over and position effect. Bridges (1936) using salivary gland chromosomes showed conclusively that the bar gene was, in fact, a duplication of a five-band segment in the X-chromosome.

Studies of salivary gland chromosomes indicate that there are repetitions of sections of genes in a number of places. These are called as 'repeats' and they are produced due to duplications. During evolution, genes in the different repeated segments become differentiated in the various directions by a process of mutation, because when duplicate genes are available, one is free to mutate. So we see that duplications have an important role in the evolution of new species.

#### Types of Duplication

There are three basic types of duplication. The segment which has been added has the same gene order as in the original segment. It is also adjacent to the original segment in the same chromosome. In the reverse tandem duplication, although the segment is adjacent to the original segment, genes are in the reverse order. In the displaced duplication, the segment is inserted into a different chromosome.

> Tandem duplication ABC ABC DEF ABC CBA DEF Tandem reverse duplication Displaced duplication LMN ABC OPR

It is obvious that the effects of duplication will be in general as well as on viability less harmful as compared to those of deficiency. So duplication can persist relatively longer. Individuals carrying duplications, however, exhibit several abnormalities in bodily characters. Such abnormalities have been used to identify the carriers of corresponding duplications.

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#### Inversions

Immediately after the discovery of linkage and recombination in Drosophila melanogaster, it was observed that there were some strains which contained what were called C-factors. These factors were supposed to reduce or suppress the recombination of genes in a certain chromosome in females heterozygous for these factors. It was Sturtevant (1926) who explained this curious behaviour. He observed that one of the C-factors which suppressed recombination was in the right limb of the chromosome III, i.e. from the kinetochore to the right end, specifically from the loci of genes Dft and p to Ca and Mg. It was in fact, a case of inversion of a section of this chromosome. A heterozygous inversion causes suppression of recombination. This will be clear when we consider the cytological effects of inversions.

Sometimes, a chromosome breaks at two points and the central piece gets inverted. For example, consider a chromosome with the gene order abcdefgh and assume that it gets broken between b and c and between f and g. The central piece rotates through 180° so that the gene order now becomes abfedegh (Fig. 23.4). fede, therefore, represents the inverted piece as regards the gene order.

Let us now consider how synapsis takes place between chromosomes in case of an inversion. The chromosomes synapse in such a manner that corresponding genes lie exactly opposite one another. This often leads to the formation of a loop as will be clear from Fig. 23.5, where the capital letters indicate the original sequence and the small letters the inverted chromosome.

You will see from Fig. 23.5 that the synapsis is remarkably precise. There are more complex arrangements when there are inversions within an inversion. However, in such cases also, the general principle of having corresponding genes opposite each other is followed.

An organism may be homozygous or heterozygous for inversion (Fig. 23.6). They may be homozygous for the standard order of parts in the chromosome. The standard chromosome order refers to the usually established order within the group of organisms. As regards inver-

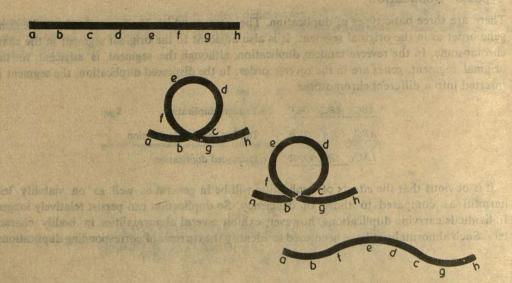


Fig. 23.4 A possible way of origin of inversions.

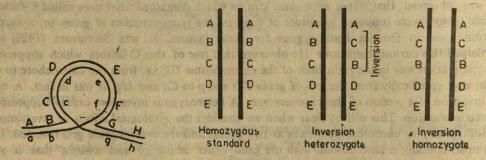


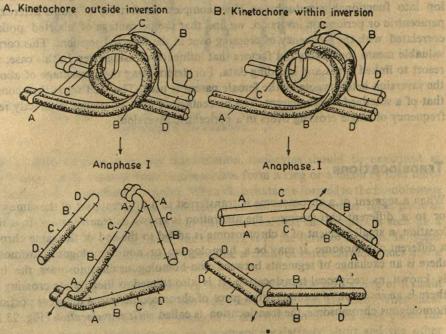
Fig. 23.5 Pairing in an inversion heterozygote, Loop formation has taken place in the region of inversion.

Fig. 23.6 Comparison of chromosomes with standard order in a chromosome pair with inversion heterozygote and homozygote pairs.

sion homozygotes, their behaviour is normal as regards cytological activities. If during evolution, an inversion type increases to a large extent in a population, there is a possibility of its being regarded as the standard type.

Inversions probably arise in various ways. Some inversions most probably arise as indicated in Fig. 23.4. A chromosome may form a loop with breaks occurring at the point where the chromosome intersects itself. In such a case, a single unit would be formed because the sticky ends always adhere to each other. If this occurs, there is no need in case of the recently broken ends to unite in the original combination. When they do not unite and find on the other hand, a new partner, the result is an inversion.

We can distinguish between paracentric and pericentric heterozygous inversion on the basis of the cytological consequences. In case of the former, crossing over takes place in the non-inverted region while in the latter case crossing over is in the inverted region. When it is not possible to examine the pachytene chromosomes for detecting the inversion loop, the formation of a bridge (Fig. 23.7) and fragment should be accepted as an evidence for the paracentric inversion. In both types of inversion, the products of a single crossing over in meiosis are two chromosomes with one deficient chromatid and a standard or an inversion chromatid (Figs. 23.8 and 23.9).



Configuration showing effects of crossing over within the inversion loop in case of paracentric (A) and pericentric (B) heterozygous inversion.

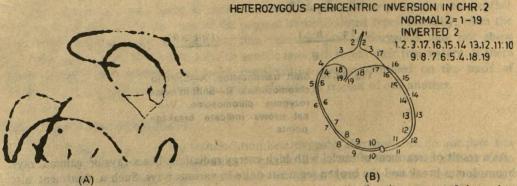


Fig. 23.9 A—Photomicrograph of heterozygous pericentric inversion in chromosome 2 in maize B-Diagram explaining A (Courtesy: Dr. S.H. Tulpule.)

You will now see that an inversion does not actually suppress crossing over cytologically. But genetic recombinations in the inversion segment are effectively suppressed since single crossover chromatids carrying these recombinations are not viable.

Being of a duplication or deficiency type, they do not either function in fertilisation or are unable to produce viable zygotes. It has been observed that the presence of an inversion exerts a powerful influence so as to reduce recombination immediately outside the inverted segment as well as within it.

In case of plants, spores containing deficient chromosomes usually abort and do not develop into functional gametophytes. If we compare the fertility of plants heterozygous for a paracentric or pericentric inversion, we find that the percentage of aborted pollen grains is correlated with the frequency of crossing over in the inverted segment. This correlation is a valuable means for finding out factors that influence crossing over. In this case, we need not resort to linked genes and breeding data. For determining the percentage of aborted ovules, the inversion heterozygote as the female parent is used. The seed set is then compared with that of a standard female parent. The percentage of aborted ovules is directly related to the frequency of single crossing overs in a pericentric inversion.

#### **Translocations**

When a segment of a chromosome is transferred to a different part of the same chromosome or to a different chromosome, the aberration is known as translocation. In a simple translocation, a small segment of a chromosome is added to the end of the same chromosome or a different chromosome. It may be a homologous or non-homologous chromosome. When there is an exchange of segments between non-homologous chromosomes, the translocation is known as reciprocal translocation. It is also termed as illegitimate crossing over. When there is an insertion of an interstitial piece of chromosome into a different portion of a non-homologous chromosome, the translocation is called shfit translocation (Fig. 23.10).

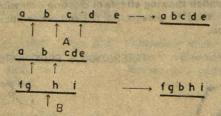


Fig. 23.10 Shift translocation. A—Shift in chromosomes. B—Shift in heterozygous chromosome. Vertical arrows indicate breakage points.

As a result of treatment of nuclei with high energy radiations like x-rays or gamma rays, chromosomes break and the broken segments unite in various ways. Such a treatment also increases the translocation frequency.

#### Cytology of Translocation

This is complicated. We shall deal only with reciprocal translocations which are most frequent and important in genetic systems. There are three basic chromosome types: (i) standard type, which is a non-translocated type, (ii) translocation homozygotes, and (iii) translocation heterozygotes (Fig. 23.11).

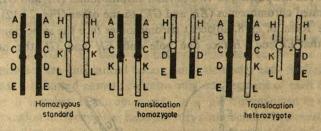


Diagram showing standard, homozygous and heterozygous translocation.

For detecting a heterozygous reciprocal translocation, meiocytes should be examined at diakinesis or metaphase-I. Usually, four chromosomes form a ring or a chain of four chromatids referred to as the interchange complex. When chiasmata are formed in the homologous segments of the cross-configuration, an association of four chromosomes takes place at metaphase-I. If each arm of the cross is with at least one chiasma, the complex is seen as a ring. When one arm is devoid of a chiasma, the complex is in the form of a chain. In some instances, the terminalisation of chiasmata can cause a chain of three chromosomes and a univalent or two pairs of chromosomes.

In maize, for determining the frequency of pollen mother cells with a ring or a chain interchange complex, the relative lengths of the translocated segments are used. When most of the cells exhibit a ring complex, one segment is short. When the cells have either the chain complex or two pairs of chromosomes, one segment is relatively short and the other segment very short. These relations can also be used in other species to determine the relative lengths of the translocated segments of interchange complexes.

Translocations may not show obvious cytological characteristics. Their pairing is regular at meiosis. Even the transmission of chromosomes from one nuclear generation to another may be as simple as in the case of the original untranslocated types. Actually, when the translocated segments are about the same size, translocation homozygotes may not show the aberration at all. Of course, suitable genetic tests will reveal their true nature. If there are recognisable chromosomal landmarks, translocation can be identified on the basis of changes in the physical relationship of their landmarks in respect of one another.

#### Translocation Heterozygotes

During meiosis, in case, of the translocation heterozygote, chromosomes do not pair but form a cross-shaped 4-armed configuration (Figs. 23.12 and 23.14). The arms radiate from a common point. Each arm consists of a pair of homologous segments. This is the only possible configuration where segments of all chromosomes can pair throughout their length. When chromosomes separate at their midregions owing to terminalisation of the chiasmata, they remain attached for some time at their ends. Because of this, a circle is formed (Figs. 23.12 and 23.13). The normal chromosomes (EF and GH) alternate with the translocated ones (EH and GF) within the circle. If the circle gets twisted in the configuration of 8 (Figs. 23.12 and 23.13), alternate chromosomes of the circle face the same pole at metaphase. This results in the passing of two normal chromosomes (EF and GH) to one gamete and two translocated chromosomes (EH and GF) to the other (Fig. 23.12). If the circle does not twist in the form of an 8, adjacent chromosomes face the same pole at metaphase. It may be possible that EF and GH face one pole and EH and GF the other (Fig. 23.12).

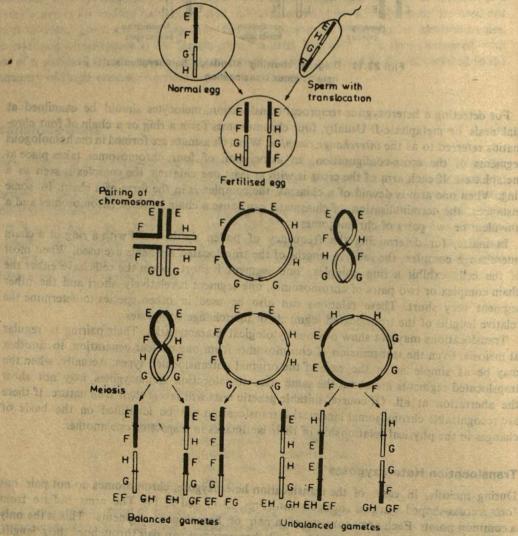


Fig. 23.12 Translocation heterozygote.

Consequently, EF and GH would pass into one gamete and EH and GF to the other. On the contrary, EF and GF would face one pole and EH and GH the other, with the result that two corresponding classes of gametes would be produced (Fig. 23.12).

If two adjacent chromosomes in a ring pass to the same pole, the gametes that are formed contain one normal (EF) and one translocated (GF) chromosome. However, the segment H is absent in the gamete with EF and GF chromosomes, while it has a double dose of the segment F. In other words, the gamete has a deficiency for H and a duplication for F. Theoretically, the translocation heterozygote ought to form six classes of gametes. Of the two classes, one contains two normal chromosomes (EF and GH) and the other two translocated chromosomes (EH and GF). So these are balanced gametes. In the case of the rest of the four classes, the gametes are unbalanced because of deficiencies and duplications. It should be remembered that a balanced gamete contains only one segment of each type.

When there are a number of chromosomes in a nucleus involved in translocation, the cytology of translocation heterozygotes becomes very complicated. In Oenothera lamarckiana, a single ring is formed at meiosis. Similarly, a more or less complex ring formation has been reported in Datura stramonium, onion, Paeonia and many other plants.

#### **Genetics of Translocation**

Translocation causes alteration in the linkage relationship of genes contained in the exchanged chromosomal segments.

Translocation also results in semi-sterility. This is a characteristic feature of many translocation heterozygotes. If a plant is heterozygous for a single reciprocal translocation, chromosomes from the meiotic translocation configuration, go two by two randomly to opposite poles. Two-thirds of the resulting spores will be non-functional because of the deficiency and duplication (Fig. 23.15). However, random separation by twos of members of a translocation ring is rare. Generally, the kinetochores of chromosomes separate disjunctionally with the homologous kinetochores passing to opposite poles. Hence, out of the six types of gametes shown in Fig. 23.15 (upper portion), the two at the left are seldom found. Hence, about 1/2 rather than 2/3 of the gametes of translocation heterozygotes appear to be formed containing duplication and deficiency thus resulting in semi-sterility.

In maize, because of semi-sterility, gaps are found in the unfilled ear shown in Fig. 23.16. These gaps indicate the position of about half the number of ovules which were aborted. In this case, the semi-sterile maize plant was pollinated.

#### SUMMARY

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- 1. Changes within the individual chromosomes are called chromosomal aberrations. Chromosomes can undergo changes. If they are broken, their normal structure gets disrupted. McClintock has found that such broken chromosomes undergo breakagefusion-bridge cycle in meiosis. Chromosome aberrations can occur simultaneously through such a cycle for sometime.
- 2. Chromosome aberrations are of four types: (i) deficiencies of deletions, (ii) duplicaverse tanded auphositons; pau tions, (iii) inversions, and (iv) translocations. me, in general, less harmigh as compared to these of do

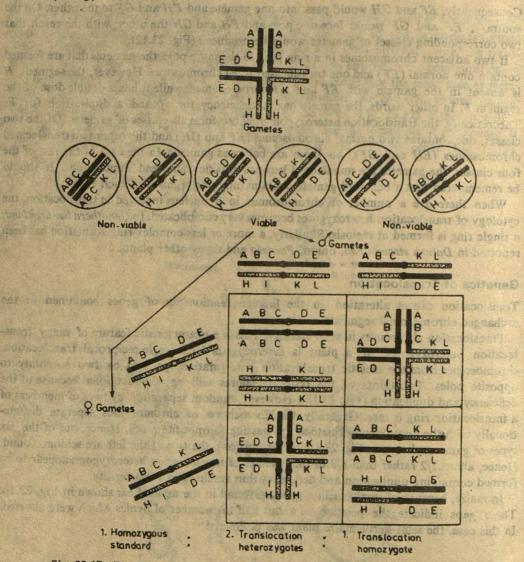


Fig. 23.15 Formation of gametes and the results of self-fertilization in case of a translocation heterozygous plant.

- 3. Deficiency is the simplest type of chromosomal aberration in which the chromosome is devoid of a short segment. It often exhibits pseudo-dominance. Deficiencies have been used to locate the actual physical position of genes in chromosomes and to prepare cytological maps.
- 4. The Bar mutant in *Drosophila* is a good example of duplication. There are repetitions of sections of genes in a number of places. There are (i) tandem duplications, (ii) reverse tandem duplications, and (iii) displaced duplications. The effects of duplication are, in general, less harmful as compared to those of deficiency.

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- 5. Sometimes, a chromosome breaks at two points and the central piece gets inverted. This is referred to as inversion. An organism may be homozygous or heterozygous for inversion. The behaviour of inversion homozygotes is normal as regards cytological activities. In case of paracentric heterozygous inversion, crossing over takes place in the non-inverted region, while in the case of pericentric heterozygous inversion, crossing over is in the inverted region. In both the cases, the products of a single crossing over in meiosis are two chromosomes with one deficient chromatid and a standard or an inversion chromatid. Genetic recombinations in the inversion segment are effectively
- 6. When a segment of a chromosome is transferred to a different part of the same chromosome or to a different chromosome, the aberration is known as translocation. There may be simple translocation or reciprocal translocation. In case of the latter, there may be a homozygous or heterozygous type of translocation.

7. Translocation causes alteration in the linkage relationships of genes contained in the exchanged chromosomal segments. It also results in semi-sterility which is a characteristic feature of many translocation heterozygotes. TOTAL SOUTH SELECTION OF THE SELECTION O

### FURTHER READING

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Begg, C.M.M. (1959), An Introduction to Genetics, The English University Press, London. Chandrasekharan, S.N. and S.V. Parthasarathy, (1953), Cytogenetics and Plant Breeding, P. Varadachari

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Garber, E.C. (1972), Cytogenetics-An Introduction, McGraw-Hill, New York. Hexter, W. and H.T. Yost, Jr. (1976). The Science of Genetics, Prentice-Hall of India, New Delhi (Reprint,

Srb, A.M., R.D. Owen and R.S. Edger (1965), General Genetics, 2nd edn, Eurasia Publishing House,

From the evolutionary characters the pecies powers after heart chromosome numbers are some server of other a france of them thought with the community of the state of the first out the common of are named his have coplete from the sand a lawer other by a direct increase of the

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Swanson, C.P. (1957), Cytology and Cytogenetics, Englewood Cliffs, New Jersey.

We have already seen that an individual containing two sets of chromosomes is called a diploid. This doubling of chromosomes is due to the fertilisation taking place between the male and female gametes, each contributing one set of chromosomes. Genes that are carried in the chromosome are relatively stable. Occasionally, however, they mutate. In addition to gene mutations, changes could occur in the number of chromosomes resulting in increase in the number of chromosomes. Such a process is known as polyploidy, and cells possessing more than two sets of chromosomes are called polyploid cells. These cells are formed when the process of chromosome duplication gets out of phase with the cell division process. For example, when chromosomes undergo an extra duplication a tetraploid cell is formed, and with two duplications, an octoploid cell results.

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The number of chromosomes in one set is called the basic number. There are many plant species belonging to a given genus possessing chromosome numbers that are multiples of the basic number; for example, the wheat genus Triticum. The most primitive species are diploid (7 pairs of chromosomes) while other species are tetraploid (14 pairs) and hexaploid (21 pairs) (Fig. 24.1; Table 24.1).

Table 24.1 Chromosome Number of the Whoat Species

| Species Species          | Chromosome number n (gamete) | 2n (somatic tissue) |
|--------------------------|------------------------------|---------------------|
| Triticum monococcum      | 7                            | 14 (7): 1 (0)       |
| T. durum, T. dicoccoides | 14                           | 14 (Diploid)        |
| T. vulgare (aestivum)    |                              | 28 (Tetraploid)     |
| - Monte (wedstrum)       | 21                           | 42 (Hexaploid)      |

From the evolutionary standpoint the species possessing the higher chromosome numbers are considered more advanced than those having lesser numbers. Plants with higher numbers are assumed to have evolved from those having a lower either by a direct increase of the numbers or by an increase after hybridisation with other species.

In 1917, the Danish genetist Winge noted that there is frequent occurrence of different multiples of a single basic chromosome number among related groups of plants. He suggested on the basis of his observations that the process of reduplication of chromosome complements takes place frequently in the evolution of taxonomic species; for example, polyploid series in wheat (*Triticum*), cotton (*Gossypium*), potato (tuberous species of *Solamum*) and tobacco (*Nicotiana*). Many cultivated plants such as wheat, rice, and several vegetal-le and ornamental plants are polyploids. It is estimated that about 30 to 45% of the angiosperms are polyploids. Among dicotyledons, a high frequency of polyploidy has been reported in

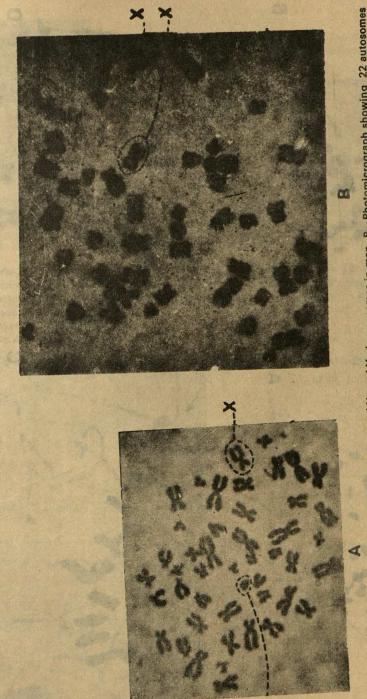


Fig. 22.2 A-Photomicrograph showing 22 autosomes and X- and Y-chromosomes in man. B-Photomicrograph showing 22 autosomes and two X-chromosomes in woman. (Courtesy: Prof. G.B. Deodikar, Ex-Director, MACS, Pune).

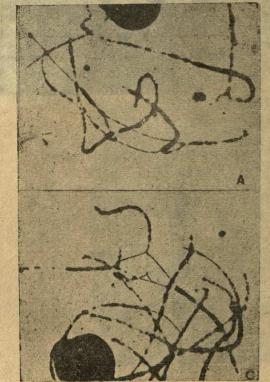


Sex determination in Coccinia indica. A-Somatic metaphase chromosomes (2n=48) in the female tetraploid plant. B—Somatic metaphase chromosomes in the male tetraploid plant. XXXY configuration is shown by an arrow. C—Meiotic metaphase in the trisomic male plant. XXY chromosomes are indicated by an arrow. D—A branch of trisomic male plant bearing male flowers in the leaf axil. (Courtesy: Prof. R. P. Roy). Fig. 22.6



Fig. 23.7 Bridge formation during anaphase of meiosis in Coix in case of paracentric inversion. (Courtesy: Dr. A.B. Sapre).

Fig. 23.13 Metaphase-I of meiosis in Coix (2n=10). Four chromosomes have formed a ring in A and a figure of 8 in B due to heterozygous translocation. (Courtesy: Dr. A.B. Sapre).



HETEROZYGOUS TRANSLOCATION BREAKS
IN 9L & 4L

95

45

9L

HETEROZYGOUS TRANSLOCATION BREAK POINTS IN 9L & 4L

B

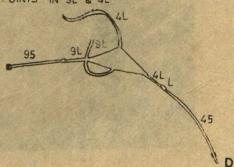


Fig. 23.14 A to D—Heterozygous translocation between chromosome 9L and 4L in maize. B—Diagram to explain A. D—Diagram to explain C. (Courtesy: Dr. S.H. Tulpule).

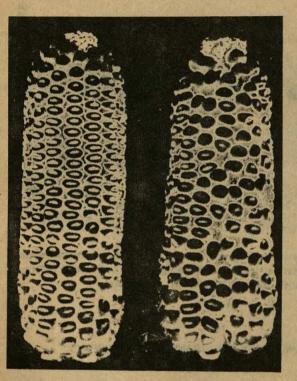


Fig. 23.16 Visible effect of semisterility in maize. The ear on the left side is normal, while that on the right side is of a plant with heterozygous translocation.

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Fig. 24.6 C-mitosis in Chlorophytum tuberosum. (Courtesy: Dr. C.D. Chowta).

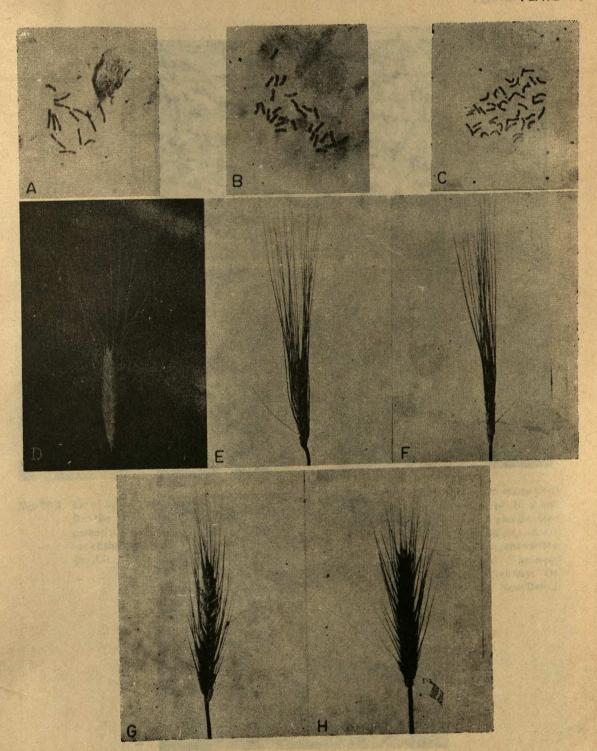
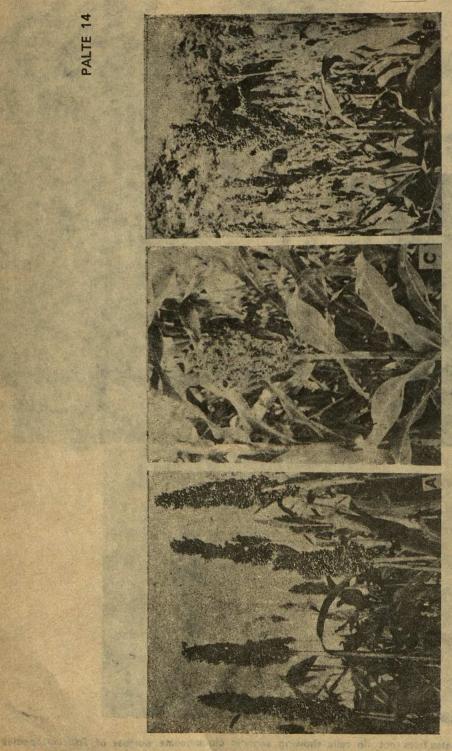


Fig. 24.1 Metaphase plates from root \_tip cells showing somatic chromosome number of *Triticum* species A—T. monococcum (2n=14). B—T. durum (2n=28), C—T. aestivum (2n=42). D—Ear of T. monococcum. E—(side view) and F (front view)—T. durum. G—(side view) and H—(front view)—T. aestivum. (Courtesy: Prof: G.B. Deodikar and Dr. V.P. Patil, MACS, Pune).



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Fig. 33.1 A—Female parent (male sterile) CK 60A. B—Jowar plant (CSH.1) produced as a result of hybridization between A and C. C—Male parent IS. 84. ( Courtesy: Dr. J.R. Kakade and Dr. S.B. Lal).

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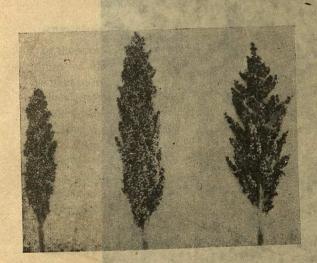


Fig. 33.2 Ear of hybrid jowar (CHS. 1) in the middle.
On the left hand side, ear of the female
parent (CK 60A) and on the right hand side,
ear of the male parent (IS. 84). (Courtesy:
Dr. J.R. Kakade and Dr. S.B. Lal).

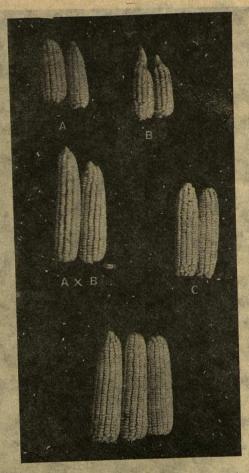


Fig. 33.3 Triple cross method in maize used in the production of Hi-starch hybrid. Upper row—A and B inbred lines. Middle row—Left side, A×B, right side, C, male parent variety, Bottom row—Hybrid between (A×B) and C. (Courtesy: Dr. Joginder Singh, IARI, New Delhi)

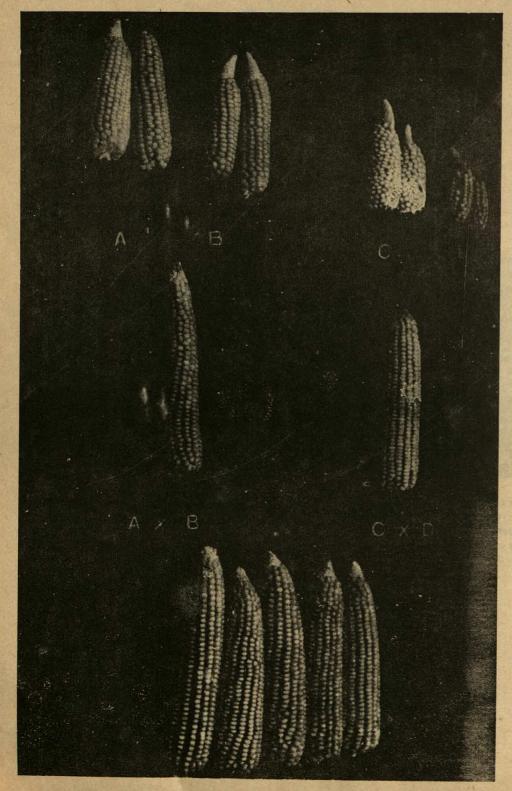


Fig. 33.4 Double cross method in maize. Upper row-A, B, C, D, inbred lines. Middle row-F<sub>1</sub> hybrid A×B and C×D. Bottom row-Deccan hybrid between (A×B) and (C×D). (Courtesy: Dr. Joginder Singh, IARI, New Delhi).

families like the Crassulaceae, Malvaceae, Nymbhaeaceae, Polygonaceae and Rosaceae. Among monocotyledons, about 75% plants are polyploids.

Polyploidy is common in plants but rare in animals. There are only a few polyploid animal species that are known; for example *Drosophila* and *Salamander*. Polyploidy for certain tissues, is, however, common to plants as well as animals. It has been observed that mammalian cells grown in tissue culture often become polyploid.

## Terminology of Ploidy

Polyploidy is classified into two main types: (i) aneuploidy and (ii) euploidy.

#### Aneuploidy

In these cases, the nuclei contain chromosomes whose members do not occur as true multiples of the basic number in the genome or genomes present, i.e. they have some chromosomes more number of times than others. An euploid plants or animals, therefore, possess incomplete genomes. An organism in which one chromosome (2n-1) of a diploid complement is absent is known as monosomic, whereas that possessing an extra chromosome (2n+1) is called trisomic. In a tetrasomic organism, there is one chromosome in quadriplicate and the others ones in duplicate (2n+2). When there are two extra chromosomes which are different members of the genome (2n+2+2), the organism is known as double tetrasomic. In a nullisomic, two chromosomes are missing (2n-2) except for which it is a diploid organism. Besides these, there are other kinds of variations of an euploidy and a similar nomenclature is used to describe them.

## Euploidy

In this case, complete genomes are involved. In a monoploid organism, there is only one set of chromosomes, i.e. one genome per nucleus. A triploid possesses three, a tetraploid four, a pentaploid five, a hexaploid six and octoploid eight genomes per nucleus, and so on. The organisms containing three or more sets of chromosomes per nucleus are usually termed polyploids. Euploids are classified into (i) autopolyploids and (ii) allopolyploids. In case of the former, the genomes are identical or very nearly so. Reduplication of the genomes within a normally diploid species results in an autopolyploid. In the latter type, genomes that make up a multiple set are dissimilar since they are derived from different species. It may be difficult to identify a polyploid organism as an auto- or allopolyploid in certain cases.

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# General Properties of Polyploids

Polyploid plants generally exhibit accentuation of characters as compared to their diploid counterparts. The increase in the number of chromosomes may affect several morphological as well as physiological characters.

#### **Morphological Characters**

1. The stem of polyploids is thick and stout.

- 2. The leaves are broader, thicker and dark green as compared to those of diploids, e.g. Aspen.
- 3. The stomatal cells are usually larger than in the diploids.

4. Hairs if present on leaves are thicker and coarser.

- 5. There is usually overall increase in size and sturdiness of the plant organs and the plant as a whole, e.g. Nicotiana.
- 6. The flower and its parts also increase in size, e.g. Chrysanthemum.

7. The pollen grains are larger in size.

8. A majority of polyploid plants have larger fruits, e.g. Baldwin variety of apple.

9. Seeds are usually larger. During germination, they develop a large cotyledonary node.

- 10. The polyploidy causes reduction in fertility. The resulting sterility may be partly due to the irregular distribution following formation of multivalent chromosome associations during meiosis. Plant breeders have exploited sterility caused by polyploidy in developing seedless varieties, e.g. seedless water melon.
- 11. There is decrease in the number of flowers.

12. Wood cells of xylem are larger.

13. Polyploid cells as well as nuclei are larger as compared with those of diploids.

#### **Physiological Characters**

1. The rate of growth of autotetraploids has been observed to be slower as compared to that of diploids.

2. There is delay in flowering.

- 3. The nectaric content has been found to increase in tetraploid tomatoes.
- 4. In the tetraploid tomatces and cabbage, there is an increase in the ascorbic acid content.
- 5. The total alkaloid content was found to be increased in the autotetraploid of Rauvolfia serpentina (Dnyansagar and Torne, 1971).
- 6. The total percentage of Na, Ca, K and Mg has been observed to be increased but there is, however, decrease in the carbohydrate and sulphur content.
- 7. In corn meal, the tetrap'oids have been found to produce 40% more vitamin than the diploids. While in the case of grapes, diploid as well as tetraploid plants have the same amount of riboflavin and pantothenic acid.
  - 8. The osmotic concentration of polyploid cells is higher than that of diploid cells.
  - 9. The water content also increases along with the increase in cell size. This increase renders polyploids less resistant to frost as compared to their diploid counterparts.

#### **Genetic Characters**

- 1. Polyploids have more than two allelomorphic genes whereas diploids contain only two allelomorphic genes.
- 2. Autotetraploids are genetically more stable than diploids because segregation in autotetraploids results in much lower frequency of recessive types than in the diploids.
- 3. The mutation rate in polyploids is relatively much smaller than that of diploids.

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#### Aneuploidy

Trisomy and monosomy are more frequent than tetrasomy and nullisomy. After the formation of the zygote, it undergoes numerous mitotic divisions. During these divisions, there may be occasional non-disjunction of sister chromosomes at anaphase, forming one monosomic and one trisomic cell (Fig. 24.2). The aneuploid cells initiate a clone of trisomic or monosomic cells which may be included in various organs depending on their capability to compete with the euploid (standard) cells. In the case of basic diploid species, the trisomic rather than the monosomic cells are likely to form an aneuploid clone in the germinal tissue. The spores or gametes which will be produced will have either the haploid (n) or disomic (n+1) chromosome number. The extent of chromosomal mosaicism in the germinal tissue determines the frequencies of these two types of gametes or spores. Chromosomal non-disjunction may take place at either meiotic division to form haploid or aneuploid species with bivalents which can give rise to functional monosomic or disomic gametophytes.

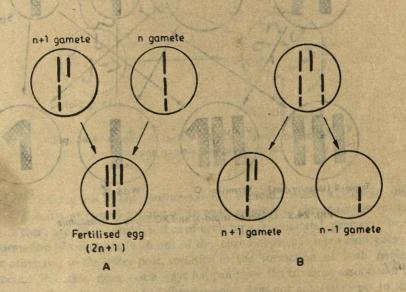


Fig. 24.2 A—Origin of a trisomic. B—Disjunction.

Recently it has been demonstrated cytologically in man that mongolism (severely related mental condition) is determined by the addition of an extra chromosome to the normal complement of 46 chromosomes (Down's syndrome). In this case, one of the smaller autosomes is in triplicate instead of duplicate. Mongolism has been found to be more frequent among children and older women. It may be that non-disjunction of chromosomes occurs more frequently as the age advances in the case of women. Figure 24.3 indicates how trisomic and XO flies arise in Drosophila. is nature and thems . It was doned have been consistent of manual by them to be the be

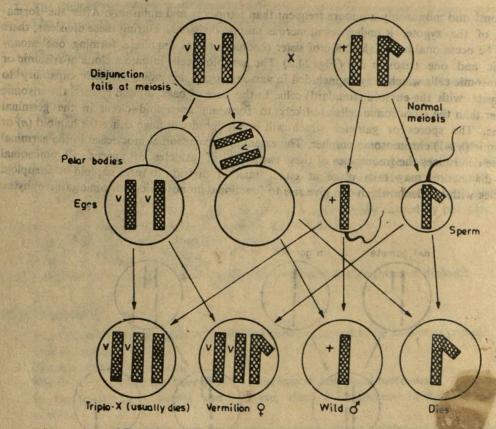


Fig. 24.3 Origin of triplo-X and XO types in Drosophila.

#### Euploidy

#### Monoploidy

A monoploid possesses only one genome where each kind of chromosome is represented only once. In the case of diploids, this is the normal condition in eggs, sperms and gameto-phytic generation in plants. So the term haploid is usually employed to describe nuclei with the gametic chromosome number.

Monoploids are generally smaller and less vigorous as compared to their diploid prototypes. In spite of these drawbacks, plant breeders are after the production of monoploids. This is perhaps because doubling of chromosomes of a monoploid gives rise to a completely homozygous plant, i.e. a pure line, which is required for the production of superior plants. In maize and tomato, monoploids have been successfully obtained by using foreign pollen to pollinate flowers.

Monoploids are characterised by their high sterility. This results because during meiosis, chromosomes do not have partners for pairing with the result that they pass to the poles in the first division at random, some going to one pole while others to the other pole. The nuclei that are formed will be deficient in one or more chromosomes. So the gametes will be non-functional.

#### Triploidy

A triploid is formed when a gamete having one set of chromosomes (Fig. 24.4) unites with a gamete having two sets of chromosomes (Fig. 24.4, 6 chromosomes). A diploid will normally form gametes with one set of chromosomes while a tetraploid will produce gametes with two sets of chromosomes. Thus, a cross between a diploid and a tetraploid will result

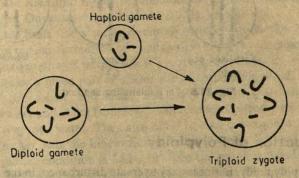


Fig. 24.4 Origin of a triploid. Two genomes come from one gamete and one genome from the

in an offspring with three sets of chromosomes, i.e. a triploid. When a triploid undergoes meiosis, the difficulty as regard passing of an equal number of chromosomes to each pole arises. Because there are three chromosomes of each type as potential partners, we should expect the formation of trivalents, with one of the chromosomes in a trivalent going to one pole and the other two to the other pole, resulting in the formation of both haploid and diploid gametes. But this does not always happen since the trivalent association is of an unbalanced nature. One of every kind of chromosomes does not necessarily go to one and the same gamete and two the other. In the first type of gametes, there will be an addition of one chromosome (n+1) while in the other type there will be one chromosome less (2n-1)(Fig. 24.5). So we see that a triploid would give rise to a few triploid offspring on selffertilisation. Since gametes other than n or 2n being non-functional, triploidy usually results in sterility. Triploids are therefore, maintained in nature through sexual reproduction.

Triploidy has been reported in grasses, forest trees, ornamental and crop plants, etc.; for example, Gravenstein and Baldwin apples or Keizerskroon tulips where the flowers are unusually large. It is found in animals like Drosophila and Salamander.

You will see that because of the irregular meiosis, triploids as a group have been rather unsuccessful in establishing themselves in nature.

Potyploidy 287

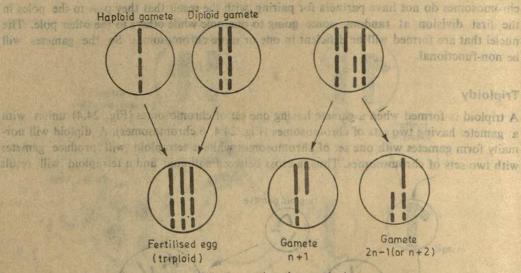


Fig. 24.5 For explanation see text.

## Artificial Induction of Polyploidy

In order to induce polyploidy, it is necessary to create disturbances in the normal synchronisation of the process of nuclear division. Several techniques have been devised to create such a condition. For example, if tomato plants are decapitated, some of the shoots that arise may be tetraploid. It has also been observed that shoots coming out near the union point in certain graft combinations are many a times, tetraploid. Randolph succeeded in inducing chromosome doubling in maize by surrounding the ear-shoot regions of the growing plants with a heating pad. In *Drosophila*, a similar effect was achieved by cold shock treatment.

A number of chemical compounds have been found to be able to induce polyploidy, e.g. acenapthene, chloral hydrate, colchicine, ethyl mercuric chloride, hexachlorocyclohexane and sulfanilamide. However, colchicine has replaced all techniques of induction of polyploidy. Methods employing colchicine have proved to be very effective in doubling the number of chromosomes in plants.

Colchicine, an alkaloid, is obtained from the plant Colchicum atumnale. Although it is very poisonous, in low concentrations it is not poisonous in case of plants. It is generally effective in concentrations from 0.01% to 1.0%. It is highly soluble in water as well as in lipoids. It is applied easily to young growing plants or germinated seeds with very little damage to them. Figure 24.6 shows doubling of 16 chromosomes in Chlorophytum tuberosum (C-mitosis) as a result of treatment of root tips with colchicine.

#### Tetraploidy and through as shieffing a diam talaura

Tetraploid plants may arise when a somatic doubling takes place and tetraploid branches are formed particularly from buds initiated from callus tissues. When the first meiotic division in the zygote fails, somatic doubling usually occurs. Tetraploids may also arise through

the union of diploid gametes. However, the probability of a diploid gamete meeting with a haploid gamete are more than in the case of two diploid gametes. Tetraploids show gigas characters and partial sterility. When a tetraploid is crossed with a diploid, a triploid is formed.

In Oryza longistaminate (wild paddy), an autotetraploid was found. This arose by somatic doubling. It had a thicker stem with darker pigments on internodes. The leaves were broader, darker and thicker. The spikelets were also larger. Although the pollen grains were larger in size, a high percentage of them got aborted. A chromosome count in the somatic cells showed that they contained 48 chromosomes as against 24 in a diploid. Most of them were observed in tetravalent associations at diakinesis in the form of rings, rods, X's and S's. At anaphase laggards and delayed splitting of univalents were also observed. The distribution of chromosomes to the two poles was variable due to irregular meiosis. A similar condition in the induced tetraploids of Rauvolfia serpentina and Sida rhombifolia was observed by Dnyansagar and Torne (1971) and Dnyansagar and Mhaske (1973), respectively.

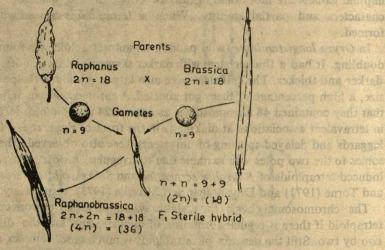
The chromosomal basis for a stable system of sexual reproduction can be available in a tetraploid if there is regular formation of quadrivalents and the disjunction from them is two by two. Still the genetics of tetraploids is much more complex as compared with that of diploids. If we consider a given pair of alleles A-a in an autotetraploid, there are two possible homozygous states, AAAA and aaaa, and three heterozygous states, namely AAAa (triplex) AAaa (duplex) and Aaaa (simplex). Different segregation ratios are obtained in the case of these heterozygous states. They also differ in each condition depending on the location of the gene whether near the kinetochore or away from it. For example, the ratio of phenotypes in the duplex condition is 35A: 1a when the gene is situated near the kinetochore whereas it is 21A: 1a in the case of genes located away from the kinetochore. because of the offect of nonlikerion or senomes. As these plants are stable and the state brigand payed at green, the should be referred as a distinct t school of the

# Allopolyploids which the cample when the condition of the

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The Russian geneticist Karpechenko made interesting genetic crosses between radish and cabbage and ultimately succeeded in obtaining a fertile and a generally true-breeding hybrid. The hybrid proved to be an amphidiploid. When two different diploid species are crossed, the hybrid obtained is a sterile one. If the doubling of chromosomes takes place in this hybrid, an amphidiploid is formed. The results of Karpechenko's work are summarised in Fig. 24.7.

The somatic chromosome number in both radish (Raphanus sativus) and cabbage (Brassica oleracea) is 2n = 18. Therefore the  $F_1$  hybrid obtained from a cross between these two species has also 18 chromosomes, 9 from the radish and 9 from the cabbage. The  $F_1$  plants differ from the radish parent. Most of their morphological characters are intermediate between a radish and a cabbage. They are highly sterile because pairing does not take place during meiosis, the genome of radish being different from that of cabbage. However, Karpechenko could obtain a few seeds which on germination gave rise to fertile plants. When they were examined cytologically, it was found that the nuclei of somatic cells contained 36 chromosomes. The behaviour of chromosomes was also regular and 18 bivalents were formed during meiosis. These results lead to the conclusion that the fertile F2 plants must have arisen from of rye-wheat parentage may be cued. It was produced by the Swedish genericus, Mantzing



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Fertile amphidiploid

Fig. 24.7 Origin of Raphanobrassica.

the union of unreduced gametes formed by the  $F_1$  plants. So the  $F_2$  plants have the ful diploid complements of the original radish and cabbage parents. The fruits of these amphidiploids are characteristically somewhat larger and the plants very robust which may be because of the effect of reduplication of genomes. As these plants are stable and different from their original parental species, they should be regarded as a distinct taxonomic entity.

Several crop plants are allopolyploids, for example, wheat (Triticum durum, 2n = 28 and T. vulgare 2n - 42), tobacco and American tetraploid cotton like Gossypium hirsutum, 2n = 52. Primula kewensis is an amphidiploid. It arose at the Botanical Gardens, Kew (England) as a result of somatic doubling of chromosomes in the sterile hybrid produced from a cross between P. floribunda and P. verticillata. As regards the spontaneous formation of an amphidiploid in the evolutionary process, the only example known is Spartina townsendii. It arose from a cross between S. stricta and S. alterniflora.

There is no foolproof method of recognising a polyploid as an autopolyploid or allopolyploid. Unless we know its origin, it is very difficult to tell its nature. Stebbins has suggested definitions of an autopolyploid and allopolyploid. They may be useful in this connection. According to him, and utopolyploid is a polyploid of which the corresponding diploid is a fertile species, while an allopolyploid is a polyploid containing the doubled genome of a more or less sterile hybrid.

# Induced Allopolyploids

It has been possible to produce allopolyploids as new crops by recombining desired characters of separate species of agricultural importance into the resulting hybrid and doubling of its chromosomes through colchicine treatment. In this way, new species can also be synthesised. An example of *Triticale* which is an amphidiploid (Secale cereale X Triticum aestivum of rye-wheat parentage may be cited. It was produced by the Swedish geneticist Muntzing

He used the following methods for producing new Triticale forms beginning with the three primary types.

1. Intercrosses between the primary types with selection in segregating generations.

2. Intercrosses between Triticum vulgare and Tricicale types. In the segregating generations, Triticale types having recombined wheat chromosomes may be obtained.

3. Pollination of  $F_1$  hybrids (wheat X rye, 2n = 28) with pollen of *Triticale*. New *Triticale* types formed in this manner are intercrossed and selection is done among the offspring. The best results were obtained by using selected rye lines.

Triticales possess the combined quality and uniformity of wheat and hardiness, and vigour and disease resistance of rye (See J.G. O'Mara, Bot. Rev., 19, 1953).

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## **Polyploidy and Evolution**

If we study polyploids in relation to evolution, we find that their rise and speed appears to be increased by the availability of new ecological places or large areas to be colonised. Such areas may be available after periods of glaciation, inundation or other surface changes. Love (1953) and other earlier workers consider polyploids as usually more tolerant than diploids of climatic extremes at high elevations and even in the arctic region.

If the diploid species occur in a stable environment with widespread distribution and possess great potential variability, then the conditions are not favourable for the evolution of polyploid species. Diploids are flexible in the evolutionary sense since they are capable of producing new types rapidly. Polyploids once developed become inflexible. Where new polyploids have originated, they have mostly evolved in the same direction as the ancestral diploids and hence, there are no radical differences in their appearance. Their formation gives an opportunity for recombination of parental characters. These recombinations enable the new species to face a new situation successfully. Hence, polyploidy to some extent revitalises former diploid species which had lost potential variability considerably and have thus become adapted to a narrow degree. This together with the possibility of rapid evolution of different polyploid forms have made polyploids expert colonisers particularly when cataclysmic changes have occurred in the environment and new areas have been opened to invasion of plants.

The distribution of polyploids is generally different from that of their diploid progenitors. In some of the groups, majority of the long-term evolutionary processes occurred at the diploid level and polyploids are perhaps of recent origin.

#### SUMMARY

1. When changes occur in the number of chromosomes and there is increase in their number, the process is known as polyploidy. Cells possessing more than two sets of chromosomes are called polyploid cells. Polyploidy is common in plants but rare in animals.

- 2. Polyploidy is of two main types: aneuploidy and euploidy. The nuclei of the former. contain some chromosomes more number of times than others and hence possess incomplete genomes. In the latter, complete genomes are involved. Aneuploids may be nullisomic, monosomic, trisomic, tetrasomic and so on. Euploids may be triploid tetraploid, pentaploid in that order. They are classified as autopolyploids and allopoly ploids. In case of the former, the genomes are identical while in the latter the genomes 3. Pollination of  $F_1$  hybrids (wheat  $\lambda$  tye. 2n = 28) with pollen of
- 3. Polyploid plants usually exhibit accentuation of characters as compared to their diploid counterparts. In aneuploids and euploids with odd number of sets of chromosomes (e.g., triploids), meiosis is irregular and often results in sterility.
  - 4. Polyploidy can be induced artificially. Colchicine is usually used for its induction. It has been possible to produce allopolyploids as new crops by recombining desired characters of separate species of agricultural importance into the resulting hybrid and doubling its chromosomes through colchicine treatment so as to produce a viable amphidiploid.
- 5. The rise and speed of polyploids in relation to evolution appears to be increased by the availability of new ecological places or large areas to be colonised. Polyploids are usually more tolerant than diploids of climatic extremes at high elevations, even in the (1952) and other earlier workers coasider polyploids as negally more to antiger pitrasloids

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# 25 Mutations

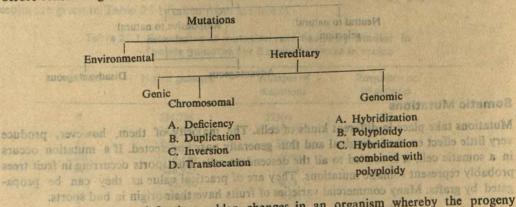
De Vries proposed the 'Mutation theory' on the basis of his observations of changes in plants of *Oenothera lamarckiana* to account for the causes of evolution. He noticed two varieties, *brevistylis* and *laevifolia*, in them. He collected seeds and obtained a total population of 54343 plants in several generations. When he screened the population, he found 843 mutants which he classified into seven types. He considered them as new species. He thought that the differences in the mutant arose by a single change in the germ and he called this sudden change mutation. Later work, however, indicated that these mutations were of a complex type. Some of them were of the nature of changes in a single gene while others were because of changes in the whole chromosome or a set of chromosomes.

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elected seen that there way be derivation of entire sets a difference (polyploidy) or loss of a set (monoploidy). Tating into condition of librar facts mutation can be defined as a sudden heritable change which is evid south under the librar heritable change which is evid south under the library conditions.

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As mutations are a part of variations, it is necessary to know their primary classification before considering them in detail. This classification is given below.



Mutations are generally defined as sudden changes in an organism whereby the progeny may show changes in size, form or composition. They are classified into those changes which occur in the chemical structure of the gene (point mutations), those causing chromosomal aberrations (deficiencies, duplications, inversion and translocations) and those involving changes in the chromosome number (polyploidy). However, at present, the concept of mutation is undergoing an extensive revision and hence a specific definition of mutation is rather difficult. Nowadays, the term is generally used to denote point or gene mutation. Chromosomal aberrations are also referred to as chromosomal mutations which we have already studied. Position effects are changes which are intermediate between gene mutations and chromosomal mutations.

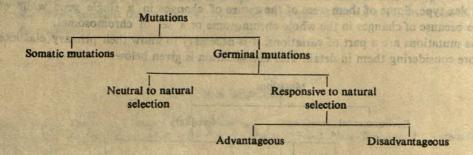
The variation which is produced due to a change in the hereditary matter may be called mutation. The process of gene duplication is usually precise. However, occasionally a

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change is observed in the duplicated gene. This is referred to as gene or point mutation since such a gene duplicated according to its changed structure. Chromosomes contain genes which occasionally undergo changes. Because of these changes, there may be loss or gain of certain genes. There may be a change in the position of certain genes in relation to others. We have already seen that there may be duplication of entire sets of chromosomes (polyploidy) or loss of a set (monoploidy). Taking into consideration all these facts, mutation can be defined as a sudden heritable change which is cytologically undetected. We shall study gene mutation in this chapter.

Plant and animal breeders have occasionally recorded the presence of 'sports', i.e. plants or animals with new phenotypes. These sports pass on the new characters to their descendants. But all of them do not represent de novo changes in the genetic material of the sport, since a recessive mutation may be carried as a hidden change for many generations and revealed only after mating of two heterozygotes.

#### Classification of Mutations



#### **Somatic Mutations**

Mutations take place in different kinds of cells. The majority of them, however, produce very little effect on the individual and thus generally pass undetected. If a mutation occurs in a somatic cell, it is carried to all the descendent cells. Bud sports occurring in fruit trees probably represent somatic mutations. They are of practical value as they can be propagated by grafts. Many commercial varieties of fruits have their origin in bud sports.

#### **Germinal Mutations**

Mutations that are most important from the hereditary point of view are those taking place in germ cells, because they can be transmitted to the next generation. Although mutation rates are very low for individual genes (because mutation occurs rately), there are so many genes that the chance of occurrence of a mutation in one or another is fairly high.

In a wild population, majority of mutations occur in the heterozygous condition. If they are recessive, they cannot be phenotypically detected. If samples of wild populations are inbred, mutant types appear due to the effect of inbreeding which increases the amount of

heterozygosity in a population. So genes which were previously heterozygous are rendered homozygous and, therefore, they can be phenotypically detected. Mutants observed in this way are found to cover a wide range of characters, morphological, physiological and biochemical. As most of the mutations are harmful, they will be of disadvantage in the homozygous condition. However, once in a while an advantageous mutation may occur, and be selected through the process of natural selection. Thus, for a species to gain an evolutionary advantage, the mutations serve as a huge store of potential variability hidden in the form of heterozygosity. In fact, mutation is the raw material on which natural selection acts.

# Mutation Rates for Individual Genes

#### Mutation Rate in Maize

Stadler (1942) made a careful study of spontaneous mutations occurring in maize. He determined mutation rates following the crossing plot method of the commercial hybrid seed planters. He grew a genetic stock dominant for a number of genes as the female parent. All tassels were removed before pollination. These plants were pollinated by pollen grains belonging to a multiple recessive stock grown in about every fifth row to provide pollen for the detasseled rows. Stadler was thus able to examine very large populations. His results are given in Table 25.1 (taken from his work).

| Table 25.1 | Spontaneous mutation rate found by Stadler in | 1 |
|------------|---|---|
|            | female gametes for 8 specific genes in maize  | 1 |
|            |   |   |

| Gene | No. of gametes | Number of mutations | Frequency per 10 gametes           |
|------|----------------|---------------------|------------------------------------|
| R    | 554,786        | 273(r)              | 492                                |
| Cl   | 265,391        | 28(CI)              | 106                                |
| Pr   | 647,102        | 7(pr)               | 11                                 |
| Su   | 1678,736       | 4(su)               | TO DESCRIPTE STORE THE PROPERTY OF |
| Ci   | 426,923        | 1(ci)               | the countries of the second con-   |
| Y    | 1745,280       | 4(y)                | hand land writeson Brosc           |
| Sh   | 2469,285       | 3(sh)               | or a see head or in over the see   |
| Wx   | 1503,744       | 0(wx)               | O sale of light                    |

#### Mutation Rate in Man

This has been studied in the case of certain abnormalities. If an abnormality is of a lethal nature, it either dies without reproducing itself or it fails to maintain its number in the population by reproduction. If it, at all, reproduces, the only way of its continuance in the population is by mutation. If a very rare and prominent abnormality occurs in a hospital, it will naturally attract the attention of the physician. If the abnormality is dominant and both the parents are not affected, then it can be inferred that the abnormality represents new mutations. In such a case, parents could not have carried a dominant gene without expressing it. Therefore, dominant abnormalities of a crippling nature are generally used in

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studying mutation rates in man. There is a kind of dwarfism in which there is bone malformation, called chondrodystophic dwarfism. Its mutation rate is about one mutant gamete in 24,000.

#### Mutation Rate in Drosophila

It has been observed that 5% of the gametes formed in a *Drosophila* fly contains a new mutation which has taken place during that generation.

It seems that mutation rates in case of man are not largely different from those of *Drosophila* and maize. They may be however, slightly higher when determined in terms of generations. If the mutation rates are determined in absolute time units like years, you will see that genes in man would mutate only about 1/1000 as fast as *Drosophila* genes because the human life cycle is about 100 times as long (30 years as compared to 12 days in *Drosophila*). It may, therefore, be concluded that the mutation rate is adjusted to conform to the life cycle.

#### **Mutation Rates for Entire Chromosomes**

Determination of the total rate for all genes is advantageous. Only the total mutation rate for assessing the total effect of mutation on the organism is required. Even when the effect of various treatments on mutation rates is to be determined, it is not possible to study individual genes since their mutation rate is extremely low. So the total rate is taken into consideration.

In 1927, Müller devised a precise method for measuring the mutation rate in *Drosophila*. The ones which are in vogue now are similar in principle to this method, referred to as the CIB method.

#### The CIB Method for Detecting Sex-linked Lethals

The C/B method is an efficient technique for the easy identification of new lethal mutations in the X-chromosome of Drosophila.

C represents a long inversion of the X-chromosome acting as a cross over suppressor and thus maintaining the integrity of the chromosome from generation to generation. I stands for a known recessive lethal located on the X-chromosome carrying the inversion. B represents the bar eye. It is used as a phenotypic marker for this X-chromosome.

Drosophila males are treated with the agent to test its ability to induce mutation. Müller used x-rays as the agent. The treated males are than mated with ClB females which are heterozygous for the ClB chromosome. From the  $F_1$  offspring, the wide bar females carrying ClB chromosome from their mothers and the 'treated X-chromosome' from their fathers are chosen. Each of them is mated with a normal male in a separate bottle (Fig. 25.1).

The bottles are then examined. If a lethal was induced on the treated X-chromosome, the  $F_1$  females mated to normal males will not produce male offspring. If, however, male flies are observed, it will mean that no lethal was induced.

#### Muller-5 Method

Later on, Müller himself modified the CIB method. The modification is called Müller-5.

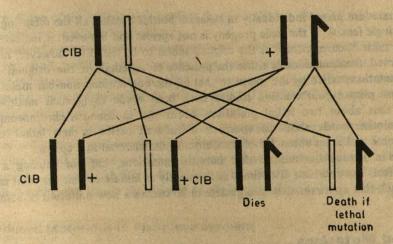


Fig. 25.1 Müller's CIB method experiment.

The Müller-5 method enables to detect whether there is a lethal mutation in the X-chromosome of a particular sperm. This mutation must have taken place during the life time of the male that produced the sperm, otherwise the zygote from which he developed would have been lethal. Let the chromosome to be tested for the presence of a lethal be represented by a dotted line (Fig. 25.2). The female used in the method is from the Müller-5 stock. In this female fly, the X-chromosome has two mutant genes,  $w^a$  (apricot eye colour) and B (bar eye). Since this is a complex inversion within an inversion, there is practically no possibility of having any recoverable cross overs.

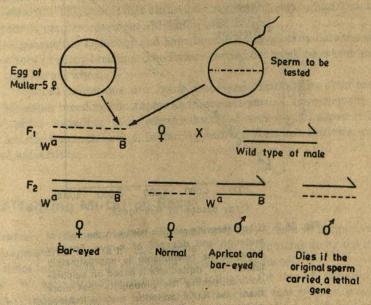


Fig. 25.2 Muller-5 method.

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 $F_1$  females are mated individually in separate bottles because all the offspring must come from a single female. If the male progeny is not apricot and bar-eyed, it indicates that they have the same X-chromosome as the original sperm to be tested. If, however, no such males are observed, the conclusion is either the presence of a lethal in the original sperm or a visible mutation carried by the sperm so that all the non-apricot, non-bar males will exhibit the mutant phenotype. Extensive studies have been made using such methods and they indicate that about two lethal mutations occur per thousand X-chromosomes per generation. In other words, about one sperm in a hundred carries a new lethal since the X-chromosome makes up about 1/5 of the chromosome material in a sperm.

Studies in *Drosophila* indicate that harmful mutations, but not causing a completely lethal effect, occur about five times as frequently as lethals so that the total rate of mutation is such that approximately one gamete in 20 carries a new mutation of some kind.

#### **Induced Mutations**

In 1927, Müller first demonstrated that x-rays increased the rate of mutation and the rate was directly proportional to the dose. If a graph is drawn, this relationship appears in the form of a straight line over a considerable range of doses (Fig. 25.3). If this essentially straight line relationship is considered as a fact, then at least two important conclusions can be derived. A specific effect is produced by a single 'hit'. If two more independent 'hits' were necessary to produce a lethal mutation, then the graph of the relationship between the density of ionisation and percentage of lethals would appear as a curve but not a

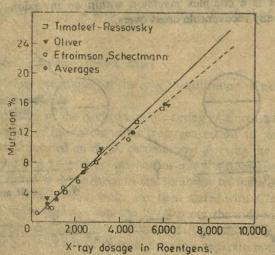


Fig. 25.3 Graph showing relation between the rate of mutation and dosages of x-rays. For low dosages, the relationship is approximately linear. The dotted line is based on calculations supposing the probability that more than one lethal mutation may be induced on any chromosome.

straight line. Some of the experiments indicate that two hits are required to have an effect on the chromosome structure by x-rays.

The amount of x-ray treatment is measured in terms of Roentgen units (R). One unit represents two ionisations per cubic micron of air, under standard conditions.

The agent inducing the mutations is called the mutagen. Ionising radiations such as x-rays, gamma rays, beta rays and neutrons act as mutagens. Ultraviolet (UV) rays are regarded as non-ionising radiation. They also induce mutations.

Many chemical substances are now known to act as mutagens, for example, mustard gas, nitrous acid, ethyl methanesulfonate (EMS), diethyl sulfate (dES), 5-bromouracil, 2-aminopurine and ethylene imine. The mutagens whose chemical nature is known are found either to interact with DNA directly or to be effective only during DNA synthesis. We shall deal more with this in the chapter on the nature of gene.

#### shipping the division of the second state of the second Use of Induced Mutations in Plant Improvement

Though spontaneous mutations occur, their frequency is extremely low and it is very difficult to detect them. So they act as deterrents to their immediate use in a breeding programme. A see the world of telephone of the see that a see that a see the see that the see th

Müller's classic paper, "Artificial transmutation of the gene" was published in 1927. It showed the way of using induced mutations in developing superior plants. Soon research was initiated in this direction so as to develop new and improved varieties by using x-rays. The Swedish geneticists Nilsson-Ehle and Gustafsson were two of the pioneers. They worked with cereals, mainly barley and succeeded in obtaining varieties with stiffer straw, a more dense head, and higher yield than the parent seed.

Certain experiments, especially those by Stadler and Sprague (1936), have indicated that gross chromosomal arrangements are either rare or absent as compared to those occurring after x-ray treatment. This means that ultra-violet rays apparently induce a higher frequency of point mutations per chromosomal aberration than x-rays.

The Russian geneticists, Delauney and Sapetin realised at an early date the potentiality of radiation as a tool in plant breeding. The former carried out work on wheat and the latter on common and durum wheats. A satellite (18) has a morting the same that a said the

Nowadays, induced mutations are being employed fruitfully by plant breeders all the world over. Mutation breeding constitutes an important part of improvement programmes of many countries including India where substantial work on this aspect is being done at the Bhabha Atomic Research Centre, Indian Agricultural Research Institute, some research institutes and universities. this life who charte made to the telephone and the country that the sent of the south and the sent

# Factors Affecting Mutation Rate and the second of the seco gallactus bare documents of the control of the dames of his

1. Temperature: The mutation rate increases with an increase in temperature within the range of temperatures that a fly can tolerate. A rise in temperature by ten degrees causes four to five times increase in the number of mutations produced.

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2. X-rays: We have already seen how x-rays influence the rate of mutation. Radiations are known to produce mutations. One may therefore think that spontaneous mutations are perhaps due to natural radiations. But the data obtained in Drosophila and mouse do not, however, support this contention. Fifty Roentgens would be required to produce as many mutations as occur simultaneously in a single generation of *Drosophila*, whereas a fly receives only about 0.004 Roentgens during this period. Similar is the case with mouse. Man has a longer life cycle, so he absorbs correspondingly more radiation during this period and has probably a larger fraction of spontaneous mutations caused by radiation. But this fraction is not yet known for want of necessary data on the rate of mutation in man from radiation. It is estimated on the basis of data obtained in the mouse that the fraction may be about 10%.

- 3. Other Higher-energy Radiations: In addition to x-rays, we have already seen that radiations of a wide range of wavelengths produce the same result. Gamma rays, beta rays and neutrons have been employed as mutagens with success. In these cases also the number of mutations produced is directly proportional to the number of ionisations in the tissue of the organism. Ultraviolet rays, said to be non-ionisation radiation generally, produce point mutations.
- 4. Chemicals: In fact, the search for chemical mutagens started earlier than for mutagenic radiations and covered a period from 1920-40. As early as 1911, McDougal made an attempt to induce mutations in plants by treatment with chemicals. The Soviet scientists, Sacharov (1931-36) and Labsov (1934) were able to induce mutations in *Drosophila* by iodine treatment, which was later (1937) confirmed by Stubbe in *Neurospora*. But the real breakthrough in chemical mutagenesis was achieved at the end of the Second World War with the reports from three different workers namely, Auerbach and Robson (1946) in the UK, Rapport (1946, 48) in the USSR and Oehlkers (1946) in Germany. Later Auerbach successfully increased the mutation rate with mustard gas. Now, many chemicals have been shown to be mutagens.

Although radiations are one of the most important tools in plant improvement programmes, their handling requires highly specialised personnel and the necessary equipment involves much expenditure. Chemical mutagens are on the other hand, easy to handle and not expensive. Their effect is so similar to that of radiations that they are called radio-mimetic substances.

The chemical mutagens include substances ranging from simple inorganic salts to complex organic molecules, and synthetic as well as natural products like alkaloids. They are classified as (i) base analogues, (ii) antibiotics and (iii) alkylating agents. Among these three, alkylating agents are by far the most important group of mutagenic chemicals. They include sulphur and nitrogen compounds, epoxides, ethylene imine, diazoalkanes, nitrosocompounds, sulfates and sulfonates. Of these EMS is the most potent mutagen.

The most precise measurements of mutation rates and the effect of chemical mutagens have been made in microorganisms. Particularly notable measurements were carried out by Novick who used the device called 'chemostat'. He found that there were some antimutagens which decreased the spontaneous mutation rate. Guanosine and adenosine substantially reduced the normal rate. His findings indicate that probably the spontaneous mutation rate is determined by the balance of mutagenic and antimutagenic substances in the cell.

#### Controlling Elements in Maize

Discovery of the presence of previously unsuspected chromosome elements involved in

controlling gene action and in inducing mutations was made by McClintock (1951, 55). She identified different controlling elements and systems of inter-related elements. They can be distinguished from one another by their distinctive ways of controlling gene action and mutation. These elements always seem to move about in the nucleus in such a manner that they may be located at one place on a chromosome and afterwards at some other place. They sometimes even move to another chromosome. An element, however does not lose its identity while moving. I miles a state of the last the

A controlling element may sometimes become associated with different genes and can modify their actions and induce their subsequent mutations. In other words, different elements may control the action and subsequent mutations of specific genes. According to McClintock, the controlling elements are normal components of the chromosome complement. They are responsible for controlling differentially the time and type of activity of individual genes. These elements have been variously referred to as activators, dissociators, enhancers, modulators, etc. The system which McClintock studied in great detail was the 'Ds-Ac' system, where Ds is a dissociator and Ac is an activator. Brink (1958) has used the term 'paramution' for this process.

The classic example of the behaviour of controlling elements is the Dotted (Dt) gene (Dt $a_1$ ) system. It was originally found by Rhoades (1936, 45). This gene caused  $a_1$  gene responsible for light coloured aleurone to mutate to A1 with the result that dark pigment was produced and black areas could be seen in an otherwise light coloured kernel. The dotted gene

had, however, no effect on other genes, not even other alleles of  $a_1$ .

The controlling elements are now also referred to as 'jumping genes'. Recent researches have shown that jumping genes are a reality (Sermonti, 1981), and are now known as transposons. These are DNA segments which can undergo excision or insertion from and into different positions of the bacterial chromosome or of DNA of plasmids or viruses. In fact, this was visualised by McClintock three decades ago and justice was done to her by awarding her the coveted Nobel Prize in 1983.

Our ideas on mutation are undergoing a profound change (Sermonti, 1981) because of new

findings. We shall deal with this aspect briefly in Chapter 30. and the upper tirely report, the belong the Street appeal of the street of the street

#### SUMMARY

1. De Vries proposed the Mutation theory to account for the causes of evolution. Mutations are defined as sudden changes in an organism whereby progeny may show changes in size, form or composition. Nowadays, the term mutation is generally used

to denote point or gene mutation.

2. Mutations take place in different kinds of cells. If a mutation occurs in a somatic cell, it is carried to all the descendant cells. But sports occurring in fruit trees probably represent somatic mutations. Terminal mutations are most important from the hereditary point of view. In a wild population, the majority of mutations are carried in the heterozygous condition. As most of the mutations are harmful, they will be of disadvantage in the homozygous condition. However, once in a while an advantageous mutation may occur and be selected through the process of natural selection.

- 3. Mutation is a rare phenomenon. Its rate is adjusted to conform to the life cycle of an organism. Determination of the total rate for all genes is advantageous. In 1927, Müller devised a precise method for measuring the mutation rate in *Drosophila*. It is referred to as the C/B method. Later on, Müller himself modified this method. It is called Müller-5.
- 4. In 1927, Müller first demonstrated that x-rays increased the rate of mutation and the rate was directly proportional to the dose. The agent inducing mutation is called the mutagen. Ionizing radiations such as x-rays, gamma rays, beta rays and neutrons act as mutagens. Many chemical substances are also now used as mutagens, e.g. mustard gas, EMS, dES, 5-bromouracil, 2-aminopurine and ethyl methane. Nowadays, induced mutations are employed fruitfully by plant breeders.
- 5. Factors which affect the mutation rate are temperature, x-rays and other high-energy radiations and chemicals. The most precise measurements of mutation rates and the effect of chemical mutagens have been made in microorganisms.
- 6. McClintock discovered in maize controlling elements involved in controlling gene action and in inducing mutation. A controlling element may become associated with different genes and can modify their action and induce their subsequent mutations. Our ideas on mutation are undergoing a profound change because of new findings.

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# 26. Cytoplasmic Inheritance

.We have seen that genes are an integral part of chromosomes and concerned with transmission of characters. This does not, however, mean that the cytoplasm, a major constituent of the cell, is not essential to the functioning and expression of hereditary characters. In fact, in a cell the proportion of cytoplasm is much more than that of the nucleus, where the chromosomes are situated. Of course, the division of the cytoplasm is not so precise as is the case with the nucleus, and, hence, the distribution of its parts is more or less random to the daughter cells formed when a mother cell divides. But it should not be forgotten that the cytoplasm is the medium in which the nucleus exists. The material concerned with the replication of the gene is transported in the cytoplasm and some of the cytoplasmic material is used in the process of replication. In spite of these facts, cytoplasm has received scant attention from geneticists as compared with the nucleus, with the result that there is voluminous information about the behaviour of chromosomes and the mechanism of heredity but, we know very little about how genes bring about changes in the developing plant or animal. In this case, the cytoplasm must have an important role. Recent researches indicate that there must be an intimate relationship between the basis of nuclear and cytoplasmic inheritance.

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When Briggs and King removed the nuclei from the eggs of a frog and replaced them by nuclei from gastrulae whose tissues were already in the process of differentiation, they observed the formation of normal tadpoles. Thus the experiment indicated that the transplant nuclei had not changed in any reversible manner. This and other experiments show that the cytoplasm itself contains sufficient inherited information required to carry out this fundamental function for a limited period. In other words, it is the bearer of at least some hereditary factors. There are a few examples known (mostly in plants) where some characters are transmitted through the cytoplasm. However, they are inherited usually in the female line only.

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#### Plastids and Plasma Genes

We already know that the green pigment chlorophyll is associated with plastids which are cytoplasmic organelles. There are albino variants whose heredity in many plants is in a Mendelian fashion. However, there are some which exhibit mostly a maternal type of inheritance.

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#### 274 Cytology and Genetics

In 1909, Correns studied inheritance in Mirabilis jalapa when he crossed green and albino varieties. He obtained variegated offspring in which areas of light and green tissues were intermingled. Branches produced from the variegated tissue were albino, green or variegated. These results indicated that plastids may have some kind of segregation which would be, of course, not a very orderly one. The work of Demerec and Anderson supports this interpretation. They observed variegation in maize. When there was occurrence of green, light and variegated seedlings on an ear, distribution of the three types was not random but there was formation of clusters. This means that variegation was because of the presence of two kinds of plastids—albino and green. These are segregated in various ways at the time of cell division and then included in the progeny.

It is generally believed that plastids have associated with them self-duplicated entities, plastogens, which are considered as forming an integral non-Mendelian part of the genetic constitution of the cell. Renner's work (1936) on Oenothera hybrids supports this suggestion. In this genus, complex translocation heterozygotes are formed. During meiosis, entire chromosome sets separate from one another as units. In O. muricata, two kinds of gametes are formed: (i) one kind with a complex called rigens and (ii) the other with a complex called curvens. O. hookeri, however, forms only one kind of gamete known as hookeri. Both these species are green. Renner made reciprocal crosses. The results are summarised below. (Only the curvens complex from O. muricata is considered here).

- 1. Q O. hookeri $\times_{\mathcal{O}}$  O. muricata  $F_1$  yellow seedlings which die
  - Q O. muricata×♂ O. hookeri
     F<sub>1</sub> Green seedlings which though survive, develop chlorotic areas later.

These results suggest that the plastid systems of the two species are adapted to their own nuclei but not even occasionally to other nuclei. Plastids (or rather plastogens) are self-reproducing particles. There is also interaction of the nucleus with these particles.

#### Male Sterility in Maize

In maize, when pollen grains fail to develop, male sterility results. This sterility is inherited through the cytoplasm. Maize plants of this type when pollinated from pollen from any strain form  $F_1$  hybrids that are pollen sterile. Crosses performed with chromosomes marked by mutant genes clearly indicate that the sterility factor is not connected with any chromosome. Recent researches point out that the factor is a submicroscopic particle contained in the cytoplasm.

Figure 26.1 shows the male sterility involving the interaction of a particular cytoplasm with genetic factors. F (fertility factor) is dominant to S (male sterility factor). So, male sterility can result only when the cytoplasmic S factor is present along the S factor of the nucleus. Female parents transmit cytoplasmic inheritance because the egg contains considerable cytoplasm besides the nucleus whereas the male gamete, i.e. the sperm, is constituted almost exclusively of the nuclear material,

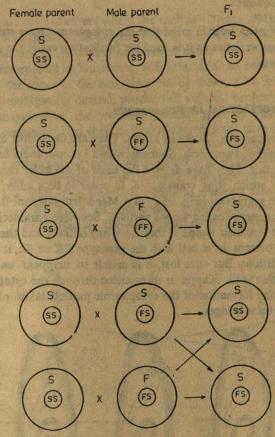


Fig. 26.1 Diagrammatic representation of male sterility caused due to interaction between cytoplasm and genetic factors. The inner circle is of nucleus and the letters therein indicate hereditary factors (genes) in chromosomes.

Letters in the outer circle indicate cytoplasmic factors transmitted through the female parent. S-Male sterility. F-Male fertile. F is dominant to S. Hence, all female parents are male fertile. On the right hand side, top and the second from the bottom row is of F1 male sterile generation. In this column, second from the top, central and bottom F1 generation is, however, male fertile.

#### Male Sterility in Onion

In onion also, there is a kind of sterility due to the interaction of genetic (nuclear) and cytoplasmic factors. This has been used in the mass production of  $F_1$  hybrids. In this

connection, it is important to bear in mind that the cytoplasmic male sterility is not as much used for obtaining new varieties as it is used for facilitating easily the crosses necessary for producing hybrid vigour.

#### Cytoplasmic Inheritance in Paramecium

Some strains of Paramecium are known as killers. They are the source of a particle called F which kills other sensitive Paramecia. Sonneborn and his coworkers carried out a series of brilliant researches on this. They have defined the basis of the killer characteristic and described the system by which it is transmitted. In order to be a killer, a paramecium must possess a gene K which segregates (Fig. 26.2) in a Mendelian fashion. It is connected with a particular cytoplasmic material called 'kappa particle'. Sensitive paramecia are devoid of this. Strains of genotype kk lose their kappa if they have any initially and then they cannot produce it. If kappa is present in a cell with the genotype KK or Kk, it continues to form it except under rare conditions. But once lost, it is unable to reappear unless more kappa is introduced from another cell. Kappa is transmitted through the cytoplasmic line within a line of descent. When the formation of the cytoplasmic particle takes place at conjugation, kappa can pass along these bridges.

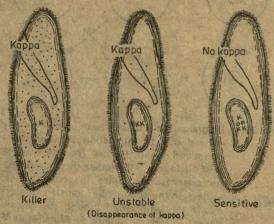


Fig. 26.2 Experiments by Sonneborn and his associates on Paramecium.

In several cases, it is known that relationship exists between a gene and a cytoplasmic particle. However, the frequency of such cytoplasmic factors is still unknown. The cases so far reported indicate that they are rarities. Therefore, the fact remains that the overwhelming majority of hereditary differences are carried by the chromosomes.

#### Coiling in Snail Shalls

The water snail Limnaea is a very good example of predetermination of traits by maternal genes. There are many species of snails in which coiling in the shell is always to the right

(dextral) while there are several others where the shell coils to the left (sinistral). There are also a few species where both dextral and sinistral individuals occur. In one of them (L. peregra), dextral coiling is dominant to sinistrality as indicated by the breeding experiments of Boycott, Diver and Garstand interpreted by Sturtevant. It is interesting to note that the trait of coiling is not determined by the genes of the individual but by those of its mother. Some of these snails which are phenotypically dextral, produce all sinistral progeny. Such individuals can be shown to be homozygous for the recessive sinistral gene by appropriate genetic tests. Therefore, it can be concluded that their dextral character must have been determined by the presence of a dextral gene in their mother (Fig. 26.3). This example indicates that it is not the character of the mother but her genes which impress upon the cytoplasm of all her eggs before maturation, a particular type of pattern which expresses itself during the early cleavage divisions of the egg. This phenomenon is known as maternal determination. When the  $F_1$ hybrids are self-fertilised, the  $F_2$  are all right coiled. In the  $F_3$ , however, a 3:1 ratio of right to left is obtained (Fig. 26.3). Hence, there is a delay in the expression of the genes till the SECTION OF SECTION OF PORT next generation. while the transfer of the course of the course of beautic

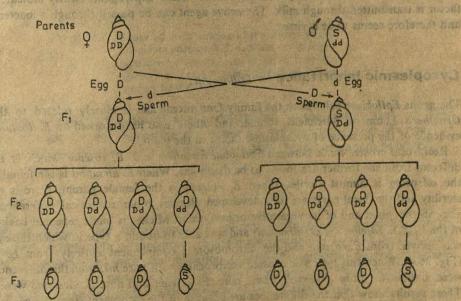


Fig. 26.3 Inheritance of dextral and sinistral coiling in the "water snail Limnaea peregra. D-Dextral phenotype. S-Sinistral phenotype. D gene for dextrality and d for sinistrality.

#### Grandchildless in Drosophila

The most extreme case of delay in gene expression is the mutant grandchildless in *Drosophila*. Females homozygous for this gene are fertile but their offspring are sterile. The factor does not, however, affect males of their offspring.

#### Cytoplasmic Factors and Infection Agents

It has been seen that there are some cytoplasmic factors which are transmitted by infection. There is a strain of *Drosophila* which exhibits a peculiar sensitivity to carbon dioxide. Genetic tests with marked chromosomes indicate that this factor is not carried on any chromosome. Generally, the sensitivity is carried by the egg and infrequently by the sperm which has very little cytoplasm around its nucleus. It was observed that a fly could be infected with sensitivity by injecting into it body fluid from a sensitive fly, thus indicating the infective nature of the factor although not by ordinary contact.

Another case is mammary cancer in mouse caused by an infective factor. It is always transmitted through the female. This was first assumed to be the case of cytoplasmic inheritance. However, a series of foster-nursing experiments revealed that mice whose mothers were from the higher cancer strain developed fewer mammary cancer if they were separated from their mother at birth and nursed by mice from a non-cancerous strain. There was, however, increase in cancer frequency in the case of mice of a low cancer strain if they were nursed by females with a high cancer strain. These experiments clearly indicate that the factor is transmitted through milk. The active agent can be passed through a bacterial filter and therefore seems to be a virus.

#### Cytoplasmic Inheritance in Epilobium

The genus Epilobium belongs to the family Onagraceae and is closely related to the genus Oenothera. Lehmann, Michaelis, Renner and others who have worked on Epilobium furnish evidence of the presence of cytoplasmic factor in the plant.

Reciprocal crosses made between Epilobium hirsutum and E. roseum which is markedly different from the former are found to be dissimilar. When E. hirsutum is the female parent, the offspring is almost sterile. When E. roseum is the female parent, there is very little sterility and the floral parts are fully developed. Besides, other reciprocal differences in the size of the plant and of its vegetative organs occur. Michaelis made repeated back-crossing of the  $F_1$  ( $\mathcal{P}_1$  E. roseum  $\mathcal{P}_2$  O. hirsutum) and was able to obtain a type in which the cytoplasm was derived from E. roseum and the chromosomal complement entirely from E. hirsutum (Fig. 26.4). When this type was crossed reciprocally with pure hirsutum the differences noted were similar to those observed when pure E. roseum and pure E. hirsutum were crossed. These results show that the differences are because of the cytoplasm since the genes are presumably identical. According to Renner and Michaelis, differences in the reciprocal crosses in the genus Epilobium are because of the production of specific changes in the cytoplasm by a particular gene combination or because of the various reactions of a given nucleus in different cytoplasms.

#### Inheritance in Rusts

Rusts are common parasitic fungi. They infect wheat and several other crop plants. The size of these fungi is generally controlled by Mendelian genes present in the nucleus. Some-

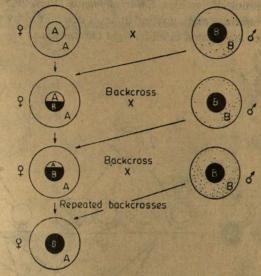


Fig. 26.4 Repeated backcrossing of the F<sub>1</sub> hybrid (Q. Epilobium roseum X & E. hirsutum) giving a type in which cytoplasm is of A type (E. roseum) and chromosomal complement B is entirely from E. hirsutum.

times, however, in case of a few rusts, it may be determined by the factors that are only transmitted through the egg indicating the non-genic type of inheritance. As rusts are fungi, they do not possess chlorophyll, so it is obvious that the factors cannot be plastids.

#### Episomes

We have already seen that transmission of an extra-chromosomal hereditary trait is independent of transmission of chromosomes and multiplication of an extra chromosomal property is not synchronised with multiplication of the chromosomal genetic materials. There are, however, certain particles possessing genetic continuity. These are known as episomes. We already know that they exist in either of two alternative states:

- 1. An integrated state in which the episome is in close association with a chromosome and multiplies in synchrony with it.
- 2. An autonomous state in which the episome multiplies independently of the chromosomal genetic material. Jacob and Wollman (1961) defined these alternative states as criteria for chromosomes.

### Non-chromosomal Genes in Chlamydomonos

Sager (1965) and her associates have carried out some fascinating work on genes outside

the chromosome in the unicellular alga Chlamydomonos (Figs. 26.5 and 26.6). This alga reproduces sexually, having two 'mating types' or sexes determined by a single chromosomal gene. The other genes are located in all its eight chromosomes.

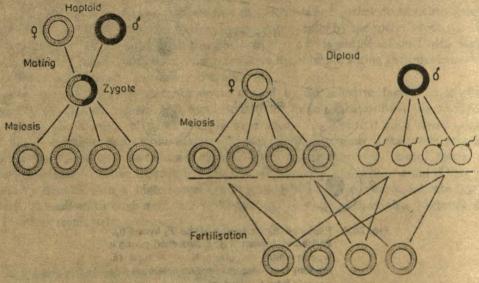


Fig. 26.5 Non-chromosomal genes show maternal inheritance. In haploid (left) and diploid (right) individuals of *Chlamydomonos*, all progeny usually get all their non-chromosomal genes (dotted rings) from the female parent. However, genes from the male parent are lost. (Redrawn after Ruth Sager, 1965).

Sager and her associates started a search for a compound which could induce nonchromosomal genes of Chlamydomonos. They discovered such a mutant accidentally. They had placed cells susceptible to the antibiotic streptomycin on the culture medium containing it. About one million cells survived and multiplied to produce streptomycin-resistant colonies. Each colony was the result of a mutation from streptomycin-sensitive (ss) to streptomycin-resistant (sr). Crossing tests indicated that although most of these mutations were chromosomal, about 10% of them exhibited a maternal inheritance pattern. They were non-chromosomal. Now the question is whether the non-chromosomal genes control a class of cell characters different from the characters determined by chromosomal genes. The answer is probably no. Sager and her associates studied 30 different non-chromosomal gene mutations and most of them were found to resemble chromosomal mutations in their effect. Even when they affect different traits, the two genetic systems seem to be apparently closely integrated in their action. It has been observed that non-chromosomal genes in Chlamydomonos follow the typical one-parent transmission pattern. The two mating types are designated as mt and mt+, with only the mt+ cell regularly transmitting its non-chromosomal genes to the progeny. Occasionally, however, non-chromosomal genes from both the parents are transmitted to the progeny where they segregate in subsequent cell divisions. The distribution of these genes is not at random during cell division. They are thetributed by means of a highly oriented mechanism. The behaviour of the mutated and normal forms of non-chromosomal genes is like alleles when they are present together in the same cells. They segregate in such a way that each daughter cell gets at least one of them. According to Sager (1965), these genes have a nucleic acid composition.

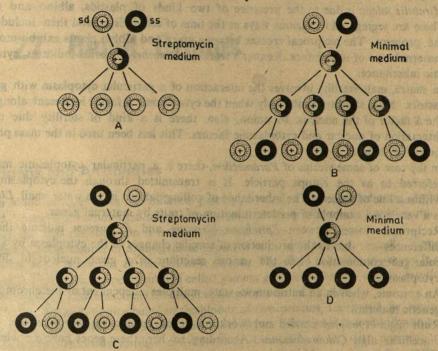


Fig. 26.6 A, B, C, D. Cytoplasmic inheritance in *Chylamydomonos*. Selection of exceptional zygotes which transmit non-chromosomal genes of the male parent. Usually a cross (A) between streptomycin-dependent ("Sd," dotted ring) female (+) and streptomycin-sensitive ("ss," black ring) male (-) cells give rise to the cells similar to the female parent. Similarly in the reciprocal cross (D), most zygotes have only as progeny-like the female parent. For selecting exceptional zygotes transmitting non-chromosomal genes from the male parent, plating of the upper cross on the minimum medium (B) and the lower cross streptomycin medium (C) is necessary. In both cases, exceptional zygotes are selected which transmit both sd and ss genes to all progeny which grow—well with or without streptomycin. Each progeny cell divides asexually to produce a clone having pure sd and ss cells (Redrawn after Ruth Sager, 1965.)

#### SUMMARY

Recent research indicates that there must be an intimate relationship between the
basis of nuclear and cytoplasmic inheritance. Some experiments indicate that the
cytoplasm itself contains sufficient inherited information and so is the bearer of at
least some hereditary factors. It is generally thought that plastids have, associated

with them, self-duplicated entities, piastogens, which are considered to form an integral non-Mendelian part of the genetic constitution of the cell. Variegation in Mirabilis jalapa is due to the presence of two kinds of plastids, albino and green. These are segregated in various ways at the time of cell division and then included in the progeny. The reciprocal crosses between green and albino plants exhibit mostly a maternal type of inheritance. Renner's work on Oenothera hybrids indicates cytoplasmic inheritance.

2. In maize, male sterility involves the interaction of a particular cytoplasm with genetic factors. Male sterility results only when the cytoplasmic S factor is present along with the S factor of the nucleus. In onion, also, there is a kind of sterility due to the interaction of nuclear and cytoplasmic factors. This has been used in the mass production of  $F_1$  hybrids.

3. In the case of some strains of *Paramecium*, there is a particular cytoplasmic material referred to as the Kappa particle. It is transmitted through the cytoplasmic line within a line of descent. The inheritance of coiling pattern in the water snail *Limnaea* is a very good example of predetermination of traits by maternal genes.

4. Reciprocal crosses between *Epilobium hirsutum* and *E. roseum* indicate that the differences are due to the production of specific changes in the cytoplasm by a particular gene combination or to the various reactions of a given nucleus in different cytoplasm.

5. An episome, when in an autonomous state, multiples independent of the chromosomal genetic material.

6. Ruth Sager (1965) has carried out work on genes outside the chromosome, in the unicellular alga Chlamydomonos. According to her, these genes have a nucleic acid composition.

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# 27. Blood Groups

#### **Antigens and Antibodies**

When blood samples from two different persons, treated to prevent ordinary clotting, are mixed, there is sometimes clumping together of individual blood cells resulting into irregular clusters. This phenomenon is termed agglutination.

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When a blood sample of an individual is placed in a dry tube, it usually clots and a yellowish fluid oozes out of the clot as it shrinks. This fluid is known as serum. It contains antibodies which are a type of protein called gamma globulin. The blood cells (erythrocytes) contain antigens which account for the specific properties of the cells.

If there is incompatibility between the bloods of two persons, there will be agglutination when these bloods are mixed in a test-tube. When a foreign substance like an antigen is introduced into the body, antibodies are formed. Antibodies react with antigens resulting in agglutination of the blood. These reactions are very specific. A person has antibodies or can produce them only against those antigens which he does not possess.

The different antigens in red blood cells are identified with the help of antibodies-proteins in the serum. Those antibodies which combine with antigens produce agglutination.

#### A-B-O Series

In 1900. Landsteiner found that when blood cells from one individual were mixed with the serum from another individual, there occurred sometimes agglutination of the cells indicating an antigen-antibody reaction. On the basis of these observations, he divided the human population into four blood groups. He observed that there was a reciprocal relationship whereby a person has antibodies for those antigens which he does not possess.

It was soon known that blood groups were inherited in a simple Mendelian fashion. The four blood groups are due to combinations of a set of three multiple alleles forming what is known as an A-B-O series. A and B mutually lack dominance with respect to each other since the heterozygote A/B is identified as a separate blood group AB and it exhibits characteristics of the homozygotes, A/A and B/B. This type of interaction between alleles is called codominance.

The blood group, genotype, antigens and antibodies and their reactions are summarised in Table 27.1. The system of nomenclature is after Landsteiner.

**Table 27.1** 

| Group | Genotype   | Reaction with antibodies |  | Blood cells | Blood serum       |
|-------|------------|--------------------------|--|-------------|-------------------|
|       |            | Anti-A                   | Anti-B   | antigens    | antibodies        |
| 0     | 010        | -                        | 10/10/20   | Nil         | Anti-A,<br>Anti-B |
| A     | A A,O A    | +                        | TABLE OF THE PARTY | A           | Anti-B            |
| В     | B/B, $O/B$ | -                        | +  | В           | Anti-A            |
| AB    | A/B        | +                        | +  | A, B        | Nil .             |

#### **Determination of the Blood Group**

A sample of blood of a person is drawn and the blood cells are mixed with serum containing anti-A antibodies. Another sample of his blood cells is mixed with anti-B serum. If it is noticed that the blood is agglutinated by both the types of sera, it is of AB group; if by anti-A, it is of A group; if by anti-B, it is of B group; and if by neither, it belongs to the O group (Fig. 27.1). The anti-A serum is prepared from the B group person while the anti-B serum from the A group person.

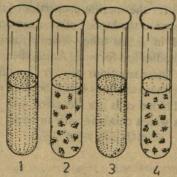


Fig. 27.1 Blood group tests. 1. When the serum of A cells and cells of A are mixed, there is no agglutination. 2. Agglutination occurs when the serum of A and cells of B are mixed. 3. There is no agglutination when the serum of B and cells of B are mixed. 4. When the serum of B and cells of A are mixed, agglutination takes place.

#### **Blood Transfusions**

It is very important to know the blood group of a person donating blood in case of blood transfusions. The transfusion of incompatible blood cells are agglutinated by the serum of

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the recipient causing blockage of capillaries and ultimately cell destruction. From Table 27.1 it will be obvious that a person belonging to the O group can be a universal donor while those belonging to the AB group can accept blood from a person of any group as his blood serum does not contain anti-A and anti-B antibodies.

#### Medico-legal Cases

On the basis of genotypes, the disputed cases of fatherhood (paternity) and other medicolegal cases are settled. For example, if both the parents belong to the O group, then their children could be only of the O group, while one parent of A group and the other of B group could have offspring of any group, because both A and B can be homozygous or heterozygous.

#### M-N-Series

In 1928, Landsteiner and Levine injected human blood into rabbits and found that they produced such antibodies which are not possessed by human beings. Human blood stimulated production of these antibodies. On the basis of the reactions of these antibodies and human blood antigens, they were able to sub-divide human population into three groups, M, N and MN. Since we do not have antibodies against cellular antigens M and N, these groups are not of importance in the case of transfusion. However, they are interesting from the point of inheritance.

In the M-N series, the alleles  $L^{\rm M}$  and  $L^{\rm N}$  are involved with no dominance. So three genotypes  $L^{\rm M}/L^{\rm M}$ ,  $L^{\rm N}/L^{\rm N}$ ,  $L^{\rm M}/L^{\rm N}$  are formed (Table 27.2).

Table 27.2

| Group | P. Patitan Wh                               | Reaction with antibodies |        | Blood cell | Blood serum |
|-------|---|--------------------------|--------|------------|-------------|
|       | Genotype                                    | Anti-M                   | Anti-N | antigens   | antioodies  |
| М     | $L^{\mathrm{M}}/L^{\mathrm{M}}$             | +                        |        | M          | Nil         |
| N     | $L^{\rm M}/L^{\rm M}$ $L^{\rm N}/L^{\rm N}$ | S DECKE MANAGE           | +      | N          | Nil         |
| MN    | $L^{\rm M}/L^{\rm N}$                       | +                        | +      | M, N       | Nil         |

Recent researches indicate that the  $L^{\mathbf{M}}L^{\mathbf{N}}$  locus is more complex. There is involved another pair of genes S and s. It is not yet known whether  $L^{\mathbf{M}}L^{\mathbf{N}}$  and S, s are separate recons or not since crossing over has not yet been observed between them.

There are reports of occasional production of anti-S and anti-s antibodies by humans of the proper genotype; for example, it has been observed that persons of s/s genotype can produce anti-S antibodies.

#### Rh Factor

In 1940, Landsteiner and Wiener discovered a different set of blood antigens while perferming tests of human blood with antisera produced by injecting rabbits with the blood of a rhesus monkey. It was found that the blood of some persons reacted with certain of these antisera even after the antibodies for previously known human antigens were removed. This means that the blood of such persons contained an antigen in common with the rhesus monkey and hence the factor was called Rh. The reactive bloods are called Rh positive (Rh<sup>+</sup>) and those which do not react as Rh negative (Rh<sup>-</sup>).

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Discovery of the Rh factor solved the cause of the haemolytic disease erythroblastosis foetalis. Normally Rh<sup>-</sup> persons do not possess antibodies to Rh<sup>+</sup> cells but may develop some because of repeated transfusions or pregnancies. An Rh<sup>-</sup> pregnant woman may produce antibodies against the antigens of Rh<sup>+</sup> blood cells of Rh<sup>+</sup> foetus. These antibodies circulate through the placenta and destroy majority of blood cells of the foetus and thus cause the disease.

The Rh factor is inherited in a Mendelian fashion. This locus is a very complex one. There has been a controversy regarding the terminology and interpretation. Wiener named it Rh° whereas Fisher proposed a different terminology in which the antigen is called D. Because of the simplicity, Fisher's system is followed here (Table 27.3).

**Table 27.3** 

| Group | Genotype        | Reaction with anti-D<br>anti-body | Blood cell<br>antigens | Bloo' serum<br>antibodies |
|-------|-----------------|-----------------------------------|------------------------|---------------------------|
| Rh+   | D/D, D/d<br>d/d | w bostone +s * Dre *c             | D<br>Nil               | Nil 99                    |

Wiener has suggested that the Rh factor is composed of multiple alleles while according to Fisher, there are three sets of linked genes. Recent researches indicate that even each of the three suggested loci has multiple alleles. Table 24.4 summarises the antisera and the equivalent genetic units as per the systems of Wiener and Fisher.

Table 27.4

| Antisera            |  | Genetic units           |          |
|---------------------|--|-------------------------|----------|
| Wiener              | Fisher                                     | Wiener                  | Fisher   |
| Anti-rho            | Anti-D                                     | R <sup>2</sup>          | CDE      |
| Anti-rh'            | Anti-C                                     | R <sup>1</sup>          | CDe      |
| Anti-rh"            | Anti-E                                     | R2 R2                   | cDE      |
| Anti-hro            | Anti-D (hypothetical)                      | COLUMN TO ROUTE A LE    | cDe      |
| Anti-hr'            | Anti-C                                     | over has stor yet been  | Cile     |
| Anti-hr"            | Anti-c in the main                         | labour base video to el | rone cdE |
| essons of sugernoty | has been observed that p                   | ti simmat voi env       | Cde      |
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Any person can possess these genes or sets of genes in such a manner that he might have any combination of two of the units. In other words, there can be 36 genotypes. Of these.

27 are identified scrologically.

It must be borne in mind that a particular antibody can be formed by a person not having the corresponding antigen. So anti-D can be formed only by a d/d person who has been immunised with cells having the antigen-D i.e. DD or Dd cells. It is estimated that the anti-D factor is responsible for most of the cases of erythroblastosis. The remaining are due to anti-C. person. In the ease of an O person, however, the donor must be of the O type

Besides A. B.O blood groun locus, there is an His A histocompatibility toons, Histocompati-

#### Other Blood Group Systems

Besides the A-B-O, M-N and Rh blood group systems, other systems have also been reported. Most of these are named after the persons in whom the factor was detected, e.g. Cellano. Diego, Dombrock, Duffy, Kell, Kidd, Lewis, Lutheran, and Stoltzfus. These are polymorphic.

In the case of the Kell-Cellano group, anti-K (Table 27.5) is usually produced in kk vomen who had given birth to erythroblastotic children.

| mixed beaute       | Table 27.5           | cels when their resp.         | o to gni |
|--------------------|----------------------|-------------------------------|----------|
| of safe to their a | Reacti               | ion with lossy send T         | unligen  |
| Genotype           | anti-K<br>antibodies | antibodies                    | End A B  |
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indry antigens show codominance alle blood groups,

#### **Blood Group Mosaics**

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In cattle sometimes, 'blood group mosaics' are found. In such cases, non-identical twin pairs have a mixture of both types of blood cells in each twin. What happens is that the blood circulatory systems during early embryogeny get interconnected with the result that the precursors of the red blood cells become mixed. In such cases of twin pairs, the female member is usually found to be sterile.

Cells of humans with mixed blood groups have been also reported. Here, A and O cells get mixed. Unlike in cattle, the female members of the twin pairs were found to be normal

and some even bore children.

# Histocompatibility and Tissue Transplants

Experiments connected with tissue transplants in the case of mice have indicated that the acceptance or rejection of tissue transplants is a genetic property and involves histocompatibility. The blood groups and the antigenic components of the cell walls in other tissues are similar. Transfusion is a kind of tissue transfusion in which we have seen that if the bloodof the same group are mixed or when the blood of O group is transfused, there is no agglutination. This is because they are histologically compatible. Similarly, in the case of transplantation of kidneys and other organs, some of the erythrocyte groups are important in relation to histocompatibility. For example, the A-B-O groups are regarded as a strong histocompatibility barrier. The donor of an organ for transplantation should not possess the ABO type nor the recipient. In other words, an AB person on the assumption that other types are compatible can accept a transplant from an A, B or O donor or from another AB person. In the case of an O person, however, the donor must be of the O type.

Besides A-B-O blood group locus, there is an HL-A histocompatibility locus. Histocompatibility antigens show codominance like blood groups.

Other Blood Group Systems

# SUMMARY

- 1. Blood cells contain antigens which account for specific properties of the cells while the serum has antibodies which are a type of protein called gamma globulin. If there is incompatibility between bloods of two persons, there will be agglutination or clumping of cells when their respective bloods are mixed because antibodies react with antigens. These reactions are very specific. In 1940, on the basis of these reactions, Landsteiner divided the human population into four blood groups, namely, A, B, O and AB. These groups are inherited in a simple Mendelian fashion.
  - 2. In 1928, Landsteiner and Levine injected human blood into rabbits and found that they produced antibodies which are not possessed by human beings. On the basis of the reactions of these antibodies and human blood antigens, they subdivided the human population into three groups, M, N and MN. These are interesting from the point of view of inheritance.
  - 3. In 1940, Landsteiner and Wiener discovered a different set of blood antigens while performing tests of human blood with antisera produced by injecting rabbits with rhesus monkey blood. The blood of such persons contained an antigen in common with the rhesus monkey and so the factor was named Rh. This factor is inherited in a Mendelian fashion. Besides A-B-O, M—N and Rh blood group system, some other systems have also been reported.

# FURTHER READING

Colle of fideless with mixed blood yeaugs have been thought or remember there. A mid. O cells

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# 28. Gene Interaction and the Work of Beadle and Tatum

# Major or Minor Characters and Genes

There are many genes which affect major or important characters or vital process. In plants, the entire process of photosynthesis could be disrupted because of the failure of a certain gene to act properly. If such a gene is missing or replaced by a mutant allele the result will be the death of the plant. Such mutant alleles are called *lethal genes*. There are some genes which affect only very minor characters. For example, in man, there is a gene which determines the presence or absence of hair on the second segment of the ring finger. In *Drosophila*, there are genes which have their slight effect on the shape of certain bristles on the wing. A great bulk of gene differences lies between these extremes and such differences, for example, genes affecting skin colour, intelligence, crop yield, milk yield, fertility and rate of growth in cattle, etc.

# **Expression of Gene-Determined Characters**

Hereditary characters may appear at any stage of development. Each gene acts at its specific times which may be any period between the zygote formation and the death of the individual. In maize and *Drosophila*, which have been extensively genetically investigated, genes have been recorded which affect almost every developmental process at different times and the same would be the case for other organisms if they were as well investigated.

# A Single or Several Genes Affecting a Character

There are genes whose effect is seen on more than one character. For example, in *Drosophila*, there is a gene for white affecting also the colour of the testes and the shape of the sperm receptacles. It is observed that blue-eyed cats are deaf. The gene 'polymorph' in *Drosophila* affects many characters like eye colour, body proportions, wing size and its vein arrangement, size and arrangement of bristles, hairs of body, shape of testes and ovary, rate of growth and fertility, and viability. A gene which has several superficial unrelated effects is known as a pleiotropic gene.

As regards several genes affecting a single character, the best example is the pigment of the eye in *Drosophila*. About 20 genes are involved in this case. In maize, more than 20 genes are concerned with the colour of the plant.

#### Normal or Wild Type

There is nothing like a gene for the eye, another for the nose, and so on. Each part of the body of an organism is affected by many genes and the effects of each gene are spread to all parts of the body.

The development of an individual is normal when all the genes function normally. If a gene is absent or has mutated to an abnormal form, there is modification of the normal effect which may be small or great. What is known about the normal gene by the geneticist is replaced by a mutant.

#### Variation in Gene Expression

There are some genes having constant expression, whereas there are others which are very variable, and in between there exist all grades. Sometimes the change in gene expression may be because of variation in environmental factors or other genetic factors which can be controlled. For instance, there is a gene 'lobe' in *Drosophila* which affects the size and shape of the eye. The effect may be such that the eye is only slightly different from normal or it may be reduced to a pin-point. Such genes are of variable expressivity whereas there are genes whose effect is constant, e.g. genes controlling eye colour.

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#### Penetrance

There are cases where a trait associated with a specific gene fails to express itself at all. In other words, such a gene has reduced penetrance. Penetrance is defined as the proportion of cases known to have the proper genotype in which the trait actually appears, i.e. a gene which is always expressed has cent per cent penetration. In *Drosophila*, the gene i makes the wing vein interrupted. About 90% of these flies possessing the genotype i/i have an interrupted wing vein while, the remaining 10% are normal. In this case, normal as well as interrupted wing vein flies possess the same genotype. This is borne out by the fact that normal flies also produce 90% offspring with interrupted wing veins. So the gene i has variable penetrance.

# **Environmental Effect on Gene Expression**

Environment may play an important role in the gene expression. The character balloon wing (wings with blisters) in *Drosophila* is affected by temperature. It is much more extreme at 19°C than at 25°C. In maize, the effect of the gene sun red depends on the amount of sunlight. Sun red plants become red in the parts receiving sunlight while those in shade are green.

There are genes whose expression is seen only in one sex, for example, early baldness expresses itself only in males. This is associated with the production of male hormones. On the other hand, there are genes whose expression is different in two sexes.

It must be remembered that the kind of environment is very important in determining the final character which a gene will produce. Not only is proper heredity essential for the expression of a character but also the proper environment. In majority of cases, complex characters like health, size, intelligence, talent, both the genetic and environmental factors are so complex and intermingled that it is not possible to separate them. However, it is possible to attribute most of the variability in a character to gene or environmental differences within the usual range of variability.

### Gene Interactions

We have already seen how genes interact with each other and modify the typical Mendelian ratios, for example, the cases of epistasis in summer squashes or skin colour in poultry.

The term 'epistasis' is also used in a most general sense to denote any instance in which the expression of two pairs of genes is different from the sum of the expressions or effects when one pair is taken into consideration at a time.

Let us again consider the case of epistasis in poultry. Both the genes C and O are necessary to produce pigment. If either is absent, the fowl is white. It may be that these two genes catalyse successive steps in a chemical process leading to pigment production as indicated below:

Some genes have the effect of altering the expression of another gene. They are known as

# Gene Control of Metabolism in Men

In 1909, Inborn Errors of Metabolism written by an English physician Garrod, was published. Hereditary defects in body chemistry were dealt with in this book. As early as 1909, Garrod had thought of specific chemical reactions controlled by genes -- a concept regarded as modern. Unfortunately, his contemporaries did not appreciate it very much. In this respect, he was like Mendel and many other pioneers of science.

Garrod studied genetic abnormalities in man. Of the heritable diseases considered by him, there is one called alkapionuria. In this disease, the urine of the affected person becomes black on exposure to air. The disease is inherited as a simple recessive. The blackening of urine is because of the presence of homogentisic acid in the diseased person. In a normal person, the acid is broken down into simpler substances. This reaction takes place under the control of an enzyme which occurs in the liver of normal persons but appears to be absent in persons suffering from alkaptonuria. Homogentisic acid is derived from amino acids, phenyl alanine and tyrosine.

A disease called phenylketonuria results when phenyl alanine is not properly metabolised. The affected persons are always feeble-minded. In other words, there is some connection between the ability to utilise phenyl alanine and mental development. Phenylketonuria is

inherited as a simple recessive.

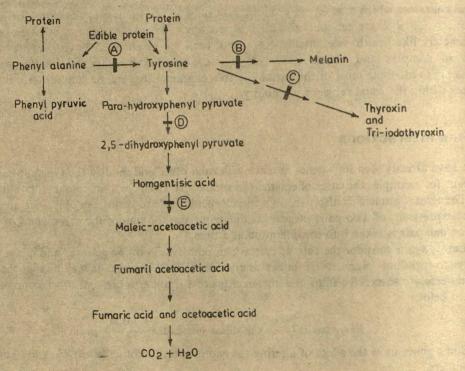


Fig. 28.1 Metabolism of phenyl alanine and tyrosine in man. Letters A, B, C, D and E indicate metabolic blocks described in the text. A—Phenylketonuria. B—Albinism. C.—Goitrous cretinism. D—Tyrosinosis. E—Alcoptonuria. (Adapted from Harris, Human Biochemical Genetics, 1959).

The colour of the skin, hair and eyes of all mammals is due to a dark brown pigment called melanin. It is derived from tyrosine through several individual chemical steps under the control of specific enzymes. When this reaction fails to take place, *albinism* (absence of melanin) results.

It has been seen that persons having a normal gene at the locus for phenylketonuria are capable of converting phenyl alanine into tyrosine, which is one of the precursors. These biochemical reactions are indicated in Fig. 28.1. Their close study leads to the following concepts.

- 1. Metabolism takes place stepwise so that certain compounds are converted into other compounds in orderly sequences of transformation.
- Specific steps in the chains of chemical reactions making up metabolism are controlled by specific genes.
- 3. Mutation of genes controlling individual steps in chemical reactions may result in creating blocks at various points in the pathways of metabolism.
- 4. The primary effects of such blocks may be (i) incapability to form certain compounds which are normal metabolites, and (ii) accumulation of precursor substances which are normally converted into other compounds.

The above concepts have far-reaching importance. It is therefore, necessary to affirm their validity and find out how widely they can be applied. It should, however, be remembered that the study of metabolism through genetic differences existing in natural populations has certain limitations. Fortunately, Beadle and Tatum overcame them successfully. This method of experimental approach is directly connected with the fundamental nutritional attributes in the fungus Neurospora selected by them for their work. Their method has now been widely used, particularly in microorganisms.

## Work of Beadle and Tatum

The main principle involved in the work of Beadle and Tatum is that many steps in the biochemical synthesis of various products are under the control of genes. A number of mutations were induced in order to have a large number of mutants different from the wild type. The life history of Neurospora belonging to Ascomycetes is well known. The nutritional requirements of the wild type of this fungus are very few. It requires certain inorganic salts, a carbohydrate source and the vitamin biotin. As these are absolutely necessary, they have to be supplied in what is called a 'minimal medium' which provides the only basic requirements for the wild type. If these are provided, the wild type is able to produce amino acids, many vitamins and other organic compounds essential for growth.

Beadle and Tatum used x-rays to induce mutations. Mutants so produced were mated with a wild type and then searched for abnormal types in the segregating generation. When a certain mutant failed to grow on the minimal medium, they inferred that there was a 'genetic block' in the synthesis of some biochemical compound essential for the life and growth of the fungus. The missing metabolites could be identified empirically by supplying in turn, various metabolites to the culture medium and see if growth resumed. However, the procedure is not so simple as it appears to be. It was a very complicated and ingenious technique. For this and other researches, Beadle, Tatum and Lederberg were awarded the Nobel Prize in 1958.

Neurospora is a very good genetic tool. All four of the products of a single meiosis can be observed directly. As their division is equational, an ascus contains eight ascospores (Figs. 28.2 and 28.3). After inducing a mutant and its mating with the wild type, a single

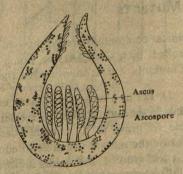


Fig. 28.2 Mature perithecium of Neurospora (diagrammatic) ...

sexual spore is isolated from the segregation resulting from meiosis. This isolated spore is then grown on a complete medium and the number of mutants is increased for further testing. Asexual spores (or mycelium) from this culture are then used for inoculating test-tubes containing different media (Fig. 28.4).

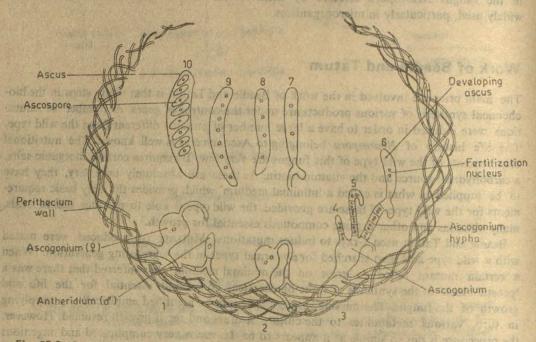


Fig. 28.3 Sexual reproduction in Neurospora (diagrammatic). The section of a part of perithecium shows one pair of sexual organs (d and Q). 1, 2 and 3 represent successive stages in sexual reproduction. Stages 4, 5 and 6 indicate different ascogenous hyphae in different developing stages and 7 and 8 stages show formation of ascospores.

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# Use of Biochemical Mutants

In Neurospora, complex chemical compounds are synthesised in a series of steps and each step is assumed to be controlled by a single enzyme. Some of these steps can be identified with the help of biochemical mutants. Let us suppose that the steps involved in a chemical

synthesis are  $A \to B \to C \to D$ . Let us also assume that a mutation destroys the enzyme and the step A to B is blocked. If the end member of the series is essential for growth, the mutant will now be unable to grow in a minimal medium. Even if A is added to the medium, it will not grow because the mutant is unable to convert A to B or B to C and C to D. Even if D is supplied, it will grow. These results indicate that the growth requirement of the mutant will not be fulfilled by any mutational element occurring before the block but can be satisfied by any one occurring after the block.

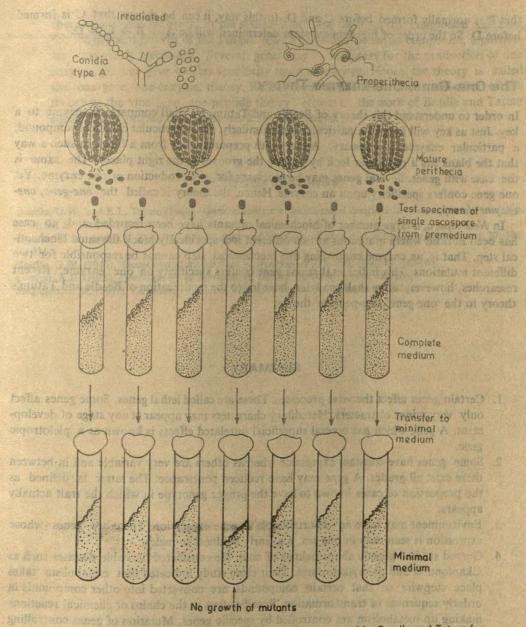


Fig. 28.4 Diagrammatic representation of technique used by Beadle and Tatum for isolation of biochemical mutants in Neurospora.

In the above example  $A \rightarrow B$  comes first. Let us assume that a mutation causes a block at 2. In such a case, the mutant will not grow if the supply of B is made to the medium. If C and D are supplied, then it will, however, grow. From these experiments, it can be inferred that B is normally formed before C and D. In this way, it can be shown that C is formed before D. So the order of biochemical steps determined will be  $A \to B \to C \to D$ .

# The One-Gene, One-Enzyme Theory

In order to understand this theory of Beadle and Tatum, we shall compare an enzyme to a key. Just as key will fit only a particular lock, similarly, for a particular chemical compound, a particular enzyme is necessary. A locksmith prepares a key from a block in such a way that the blank fits a particular lock by putting the grooves in the right places. The same is the case with genes. Several genes may be necessary for the production of one enzyme. Yet one gene confers specificity upon an enzyme. Hence, the theory is called the one-gene, one-enzyme theory.

In Neurospora, a large number of biochemical mutants have been recorded and no case has been noticed where mutations at two different loci specifically block the same biochemical step. That is, an enzyme controlling one biochemical step cannot be responsible for two different mutations. This indicates that one gene confers specificity on one enzyme. Recent researches, however, as we shall see later, have led to the modification of Beadle and Tatum's theory to the 'one-gene, one-peptide' theory.

### SUMMARY

Certain genes affect the vital processes. These are called lethal genes. Some genes affect
only very minor characters. Hereditary characters may appear at any stage of development. A gene which has several superficial unrelated effects is known as a pleiotropic
gene.

2. Some genes have constant expression whereas others are very variable and in-between there exist all grades. A gene may have reduced penetrance. The latter is defined as the proportion of cases known to have the proper genotype in which the trait actually appears.

3. Environment may have an important role in gene expression. There are genes whose expression is seen only in one sex, e.g. early baldness in males.

4. Garrod studied genetic abnormalities of man. He considered heritable diseases such as alkaptonuria and phenylketonuria. Their close study indicated that metabolism takes place stepwise so that certain compounds are converted into other compounds in orderly sequences of transformation. Specific steps in the chains of chemical reactions making up metabolism are controlled by specific genes. Mutation of genes controlling individual steps in chemical reactions may result in creating blocks at various points in the pathways of metabolism.

5. Taking the clue from the work of Garrod, Beadle and Tatum performed experiments on the fungus Neurospora. They used x-rays to induce mutations. Mutants were mated with a wild type, and they searched for abnormal types in the segregating population. When a certain mutant failed to grow on the minimal medium, they inferred that

there was a 'genetic block' in the synthesis of some biochemical compound essential for the life and growth of the fungus. For a particular chemical compound, a particular enzyme is necessary. Several genes may be necessary for the production of one enzyme, yet one gene confers specificity upon an enzyme. Hence, the theory is called the one-gene, one-enzyme, theory. Recent research has led to the modification of this theory as the 'one-gene, one-peptide theory'. However, the work of Beadle and Tatum convincingly proved that genes work through enzymes.

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# 29. Genetics of Bacteria and Viruses

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The work of Beadle and Tatum on Neurospora in 1941 gave an impetus to the genetic researches on bacteria and viruses. One of the leaders in bacterial genetics is Lederberg. He was a student of Tatum. Their discovery of recombination in bacteria proved to be a landmark in bacterial genetics. They showed that recombination of gene materials in bacteria is essentially same as in higher organisms. In fact, one can say that the higher organisms are similar in their inheritance to bacteria because bacteria appeared before the higher organisms in the evolutionary scale.

Extensive literature is now available on the work of genetics of bacteria and viruses. It has helped to a great extent in gaining knowledge of the genetic code and gene action and its application in genetic manipulation.

### Bacteria

### Sexuality in Bacteria

Until recently, an idea generally prevalent was that reproduction in bacteria was asexual and by simple cell division. However, the work of Lederberg and Tatum on colon bacillus Escherichia coli convincingly proved that some bacteria reproduce sexually. Some cells in culture behave as sex cells and unite with one another in the usual manner of sex cells. The 'fertilised' egg or zygote formed as a result of this sexual union undergoes reduction division immediately and the haploid cells thus formed then multiply by mitotic division. In other words, only the diploid cells in a bacterial culture would, as a rule, be the zygote. All the other cells would be haploid, and this is what we generally observe under the microscope and regard as bacteria. Lederberg discovered a race in bacteria which is diploid. Fertilisation in this race is followed by a large number of mitotic cell divisions prior to the reduction division.

Lederberg observed two mating types in *Escherichia coli* depending on the presence or absence of a fertility factor F. He designated male cells as F<sup>+</sup> and the female cells as F<sup>-</sup>. The F<sup>+</sup> factor possesses a peculiar property of transference of itself from cell to cell on contact. Thus, F<sup>-</sup> cells that come in contact with F<sup>+</sup> cells in the same culture become converted into F<sup>+</sup> cells. In fact, F<sup>-</sup> cells are cells lacking the F factor. We have already seen that the F factor is an episome. When it is in a free state, it has only the potential of donating a chromosome or partial chromosome to a female cell. For the occurrence of this phenomenon, integration of the particle into the chromosome is necessary. Bacteria in which the F

factor occurs as an integrated part of the chromosome are known as Hir because of the frequency of recombination.

Jacob and Wollman have shown that when conjugation takes place and the chromosome from the male cell enters the female cell, the last part to enter is always the F region and hence it often gets left out. The process of conversion of an F+ strain into Hfr by attachment of the free F particle to the chromosome is probably similar to the incorporation of the phage into the chromosome. The F particle is regarded to be circular.

It is possible to map the gene sequence by the time of the entry of genes into the female cell. A consistent map of the Hfr strain can be obtained by this procedure.

### Recombination

A remarkable result is obtained if recombination experiments are performed with a number of independently isolated Hfr strains. The order of entry of the markers into the F- from the different Hfr's is different in each case. All the orders are, however, consistent with one another if we assume that the genome of the F+ cell from which the Hfr's were obtained, is a ring and that in different Hfr's the point of entry of the male genome into the female and the direction of this entry can differ. In other words, transfer of markers by one Hfr may be in the order and direction of abcd . . . xyz, another in the order of uvwx, and still another in the order chaz . . . fed. The Hfr possesses a ring chromosome which breaks at a particular point before transfer.

Another interesting feature of this wonderful system is that all of the recombinants formed in crosses of Hfr × F- are themselves F- except for rare individuals which are in receipt of the most terminal markers from the male. These infrequent recombinants are usually Hfr's and transfer their genomes in the same sequence as did their male parent. It seems that the 'mating type' marker is always the last to enter the female irrespective of the order of entry of all other markers. And seminated the above the seminated the seminat form remaines because at a remaining and a property of the control of

exercit policy as advantages and valle he filled. The Recombination among many more loci in bacteria has been observed by Lederberg, Jacob, Wollman, Altenberg, Hayes and several others. They also found that this recombination was not at random but exhibited definite linkage patterns. The two parent types in Escherichia coli do not contribute equally to their progeny and all the genes found among the progeny are linked. This became clear only after the pioneer work of Hayes who showed the occurrence of sexual differentiation in E. coli. Certain crosses made between different strains of this organism are fertile and their recombinants are obtained. However, the other crosses are sterile and so it is not possible to get recombinants. These results show that there is some kind of differentiation in the mating types, and cells of the opposite mating type are necessary for getting recombinations. The later researches have indicated that one parent acts as a donor of genetical material while the other acts as a recipient.

Conjugation systems like the one described above seem to be infrequent among bacteria. There are, however, two other 'sexual' systems which are of more widespread occurrence and used in genetic studies. These are transformation and transduction. The said and the selections which can be referred as a statement are which

# 300 Cytology and Genetics

### Transformation

Of the methods of genetic transfer, the simplest one is transformation. Cells of a particular genotype are cultured under special conditions. They are then exposed to DNA extracted from the cells of another genotype, say, A. Up to 10% cells of progeny of treated cells get transformed into the genotype A. Transformation has been observed in the bacterial species, Bacillus subtilis, Diplococcus pneumoniae, and Haemophilis influenzae.

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### **Mutation and Selection**

Although variations in bacteria have been studied for many years, it was only recently shown that variations are caused by mutations. Bacteria exist in very large numbers and their rate of reproduction is so tremendous that they can be subjected to much higher selection intensities as compared to higher plants and animals. When a culture of several billion cells is subjected to a strong killing agent, like the antibiotic streptomycin, one or more resistant mutants are found to be present which will survive. Although their number is very low, but their rate of reproduction is so rapid that the culture will soon grow to its previous size and contain only descendents of the resistant-mutant cells. This shows the tremendous effectiveness of selection in bacteria. Most of the confusion in studies on bacterial variation was because of this effectiveness. The error was made by believing that the variations were induced by the drugs whereas it was actually a powerful selecting agent.

As regards drug resistance, the experimental distinction between the drug inducing the resistance or selecting the pre-existing resistant mutants is very minute. The fact is that the resistant culture is obtained only after drug treatment. So the question arises how could it be known that the drug did not induce the change. It was left to Luria and Delbruck (1943) to answer this question convincingly by devising two different suitable experiments to show that true mutation had occurred before the drug treatment. In both the cases, the cells resistant to the drug and their direct ancestors had never been treated with the drug.

The rapid rate of selection in bacteria has created serious problems in the treatment of bacterial diseases with antibiotic drugs. If a patient is given an antibiotic drug, it is likely that most of bacteria except a few resistant mutants will be killed. The residual mutants will, however, multiply rapidly and, thus make the drug ineffective.

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### Viruses

Viruses are much smaller than bacteria. They are the particles exhibiting the simplest form of life. There is often a doubt whether viruses are living at all. This is because some viruses can be crystallised, which is a characteristic of the non-living. In this form they appear lifeless. But when introduced into living cells, the crystal breaks up into units which multiply, a characteristic of living beings. At this level, our usual concepts of 'living' and 'self-reproducing' become inapplicable since viruses are on the border line of non-living and living.

The tobacco mosaic virus (TMV) causes leaf disease in many plants. The chemical constitution of this and some other viruses has been determined. They are composed of a protein coat and nucleic acid core which can be regarded as a chromosome. The viruses, however,

differ among themselves in relative amounts of DNA and RNA. In TMV, the nucleic acid is RNA.

Viruses are obligate parasites. They must live inside the cell. Their most distinctive feature is their incapacity to perform metabolic functions. They only use the enzyme systems and precursors of the host cell. So a virus is able to reproduce itself when its genetic material (DNA or RNA) is within the host cell. Hence, a virus can be regarded as more analogous to a chromosome than to a living organism.

### Bacteriophage

Those viruses which infect bacteria and kill them are called bacteriophages (bacteria eating) or phages. When a virus particle infects a bacterial cell, it may multiply inside the cell until the cell is finally destroyed and new virus particles are released. This is known as the lytic cycle, and the virus behaving in this manner is called a lytic virus or phage.

In some cases, the behaviour of a virus may be quite different. After its entry into a bacterial cell, it becomes a prophage. Instead of destroying the cell, the virus now becomes a regular part of it. A bacterium which carries a prophage is said to be lysogenic. Later on, the cell may lyse and release the phage. New virus particles released in this way may then infect another bacterium and begin either a lytic cycle or become prophage.

The prophage is, in fact, attached to the host chromosome. It becomes incorporated into a particular site on the chromosome. A good example of this is the phage. It is always incorporated into the chromosome near the gene for utilising galactose. This implies the existence of some similarity between a virus and a bacterial chromosome. There is strong proof that the virus chromosome is circular.

### **Mutations in Bacterial Viruses**

The relationship between Escherichia coli and the viruses that infect it appears to be such that there is always a kind of struggle going on between them. First the bacterium assumes resistance to the virus, e.g. T<sub>1</sub> due to chance mutation. Then there is a chance mutation also in  $T_1$  which is able to attach to the mutant strain of the bacterium. That is, a mutant first arises in E. coli which is resistant to the normal  $T_1$  phage and then  $T_1$  produces a mutant to which the mutant of the bacterium is susceptible.

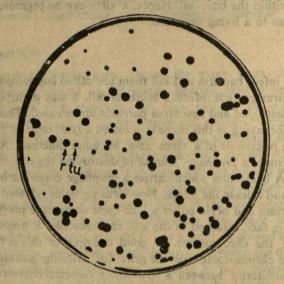
The mutant phage in the above example can infect or use as a host not only the normal strain of E. coli which is resistant to the normal phage but also the mutant of this bacterium which is resistant. In other words, the host range of a phage can be increased by a mutation. The latter may also bring about a change in the lysis caused by the phage, i.e. from slow to rapid or a specific change or some other type.

# Genetic Recombination in Bacterial Viruses

Genetic recombination has been observed in a number of different bacteriaphages. What is called as 'cross' is the infection of bacteria with a sufficiently large number of phages of two different genotypes (about 5 of each type per bacterium) with the result that practically all the bacteria are infected with phages of both the genotypes. Among the progeny phage released from the infected bacteria, recombinant types are observed (Fig. 29.1) The

### 302 Cytology and Genetics

frequency of recombination is dependent on the physical relationships between the specific markers used. Reciprocal recombinations are also observed in equal numbers. The frequen y of the recombinant type is same irrespective of the coupling or repulsion phase of the mutant markers. Genes can therefore, be mapped in bacterial viruses in much the same way as in the case of higher organisms.



Sacreclophage

Fig. 29.1 A two-gene cross in bacteriophage  $T_4$ .

Plating of a mixture of two parental types

r and ru has been done.

Many detailed experimental investigations have been made in connection with the process of recombination in bacteriophage. In the light of these investigations, it appears as follows: After a bacterium is infected, the phage gene complements or genomes which are chiefly or exclusively DNA multiply producing what is called a 'pool' of vegetative or non-infectous phage. There is interaction of these genomes in some way so as to form recombinations. Phage geneticists call this interaction as 'mating'. The matings are essentially random in regard to partner (because there are no mating types in phages). They take place repeatedly during the growth cycle.

There is little similarity between features of phage recombination and those of meiosis in higher organisms. Hence, it is a puzzle whether the mechanism of recombination during phage growth and that of crossing over in meiosis are basically similar.

Cenerio Recombination in Street It Viruses

### Transduction

Lederberg and Zinder found an altogether different kind of transmission of properties of hereditary characters through infection in the bacterium Salmonella. They termed it transduction (Zinder and Lederberg, 1952).

Transduction is the phenomenon of the transfer of genetic material from one genotype to another. The transfer is effected by a bacterium phage which acts like a 'messenger boy'.

For instance, when streptomycin-sensitive bacteria are infected with phages cultured on streptomycin-resistant hosts, a fraction of the cells which survive the infection become streptomycin-resistant. So they act as donors of this newly acquired character (Fig. 29.2).

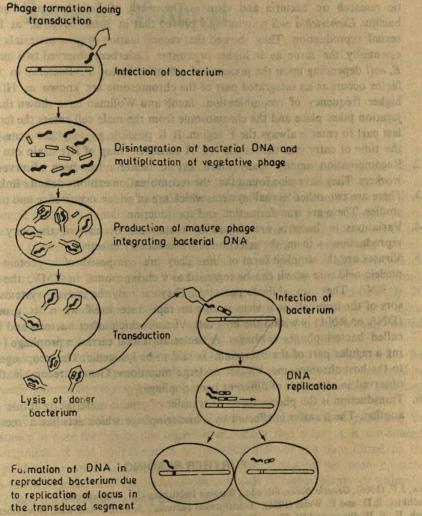


Fig. 29.2 Diagram showing different stages in transduction. (Redrawn from Singleton, Elementary Genetics, 1965).

What happens in the above mentioned case is that the phages during their multiplication in the resistant host have incorporated into their genotype some of the DNA from the bacterial host in some way. This DNA is then transferred (transduced) to the sensitive host and becomes an integral part of the genotype. Let us illustrate this by supposing that a bacteriophage leaves a bacterium of genotype A and infects another bacterium of the genotype a. The phage converts the second host into the genotype A. So it appears as if the phage carries the gene from one chromosome to the other.

### SUMMARY

- 1. The work of Beadle and Tatum on Neurospora, in 1941, gave an impetus of the genetic research on bacteria and viruses. The work of Lederberg and Tatum on colon bacillus Escherichia coli convincingly proved that in some bacteria, at least, there is sexual reproduction. They showed that recombination of gene materials in bacteria is essentially the same as in higher organisms. Lederberg observed two mating types in E. coli depending upon the presence of a fertility factor F. Bacteria in which the F factor occurs as an integrated part of the chromosome are known as Hfr because of higher frequency of recombination. Jacob and Wollman have shown that when conjugation takes place and the chromosome from the male cell enters the female cell, the last part to enter is always the F region. It is possible to map the gene sequence by the time of entry into the female cell. A consistent map of Hfr strain can be obtained.
- 2. Recombination among many more loci in bacteria has been observed by several workers. They have also found that the recombination exhibits definite linkage patterns.
- 3. There are two other 'sexual' systems which are of wider occurrence and used in genetic studies. These are transformation and transduction.
- 4. Variations in bacteria are caused by mutations. Because of the very rapid rate of reproduction in them, the selection is tremendously effective.
- 5. Viruses are the simplest form of life. They are composed of a protein coat and a nucleic acid core which can be regarded as a chromosome. In TMV, the nucleic acid is RNA. They are obligate parasites. They use only the enzyme systems and precursors of the host cell, so a virus is able to reproduce itself when its genetic material (DNA or RNA) is within the host cell. Viruses which infect bacteria and kill them are called bacteriophages or phages. A bacterium which carries a prophage (virus becoming a regular part of the bacterium) is said to be lysogenic. The prophage is attached to the host chromosome. Viruses undergo mutations. Genetic recombination has been observed in a number of different bacteriophages.
- 6. Transduction is the phenomenon of transfer of genetic material from one genotype to another. The transfer is effected by a bacteriophage which acts like a 'messenger boy.'

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# 30. Genetic Material

## Composition of Chromosomes

There is overwhelming evidence in both the sciences of cytology and genetics that the chromosomes carry the genetic material. One of the ways to determine its chemical nature is to analyse chromosomes. In fact, as early as 1907, Miescher had analysed nucleioproteins from pus cells and found that they contained phosphoric acid, pentose sugar and nitrogen bases. Recently it has been possible to isolate chromosomes from the other constituents of the cell and to analyse them. Chromosomes are not only morphologically complex but chemically also. They are composed mainly of three substances, proteins, DNA and RNA which are naturally occurring polymers. A polymer is a large molecule produced from a few small molecules which are repeatedly linked in chemical bondage. Proteins are constituted by 20 amino acids in various combinations. Different proteins may contain 100 to 1000 units. DNA and RNA polymers are usually made by repeated linkages of four simple units called nucleotides. A DNA molecule may contain even 30,000 units linked together.

We have knowledge now of chemical composition of chromosomes. However, it is to be seen which of the constituents (protein, DNA and RNA) is the hereditary material or whether the genetic information is contained in some combination. To decide this question, biological tests are to be devised.

In order to prove that a specific substance carries genetic information, it is necessary to isolate the substance in pure form from one organism and show that when it is introduced into another organism, it not only appears in the second but is also transmitted to its progeny. Though this appears to be a simple and sensitive test, it has not yet been possible to perform this test with material obtained from chromosomes of higher plants or animals. However, it has been done accurately with certain bacteria.

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# Bacterial Transformation

It is interesting to know that a study of the pestilent bacterium Diplococcus pneumoniae by Griffith led ultimately to our understanding of the chemical nature of the genetic material. Griffith carried out certain experiments in 1928 using pneumococcus bacterium D. pneumoniae. He observed that there were certain strains of the bacterium which were unable to cause symptoms of the pneumonia disease when inoculated in mice. Such strains are said to be avirulent. Pneumococcus, therefore, exists in two different phenotypes: smooth (S) and rough (R), the former being virulent and the latter avirulent. S-cells possess a capsule made

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of a polysaccharide carbohydrate while R-cells are devoid of it. Different kinds of S-bacteria can be identified on the basis of differences in the chemical composition of the polysaccharide capsule. Since each of these types is heritable, it is obvious that the capsule is a part of the genotype of the organism. It has been found that one S-cell in ten millions mutates and produces a colony composed of R-cells. R-type is also inheritable. If R-cells are calculated, a few of them mutate to S-cells. The latter are similar to original S-cells from which R-cells were derived through mutation.

Griffith inoculated a small number of living avirulent R-cells (obtained by mutation from II-S) in a mouse and found that the animal was not affected, whereas inoculation with virulent type III-S cells caused pneumonia. After this, he injected a small number of living R-cells with a large number of heat-killed III-S cells, and was surprised to observe that the mice died of pneumonia. Blood tests of them indicated the presence of III-S type cells. He concluded from this that there was transformation of II-S to III-S type (Fig. 30.1). This could not be because of mutation since R-cells were obtained through mutation from III-S cells. Griffith's results were confirmed by Dawson who worked at the Rockefeller Institute, New York. In this institute, Alloway also carried out work on transformation in pneumococcus cells which disintegrate when kept in bile salts. They appear to dissolve and a clear solution is obtained. Alloway observed that even after killing the capsulated cells by heating, these cells were able to transmit their hereditary constitution even after they had been dissolved. This means that a certain substance from the cells, and not the entire cell, was responsible for transmitting the hereditary characters of the cell.

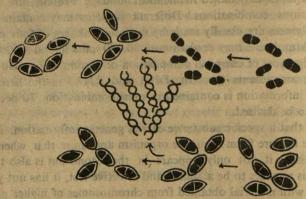


Fig. 30.1 Bacterial transformation.

As regards further transformation experiments in vitro, it was found that it was not necessary to have intact heat-killed S-cells. Some R-cells could be transformed even by using an extract of S-cells. This extract was called the transforming principle.

### Hereditary Material-DNA

The nature of the transforming principle was brought to light by Avery, McCleod and McCarty in 1944. They analysed it and proved that it was DNA. They obtained DNA

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from the extract of III-S cells. After it was highly purified they found that DNA was able to transform some R-cells to III-S cells. However, when the DNA treated with deoxyribonuclease, an which brings about disintegration of DNA, was used in the experiment, no transformation enzyme occurred (Fig. 30.2).

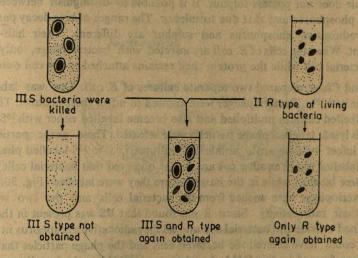


Fig. 30.2 Transformation of pneumococus bacteria in vitro.

The DNA incorporated and integrated by a recipient bacterium constitutes a small percentage of the total DNA of any given donor bacterium. Usually, transformations take place in a single gene but they may also occur in more than one gene contained in a single segment or different segments of the donor DNA.

The reaction of DNA is highly specific. If the DNA obtained from another type of pneumococcus is added to the cells devoid of capsules, the cells which ultimately multiply are of the type from which the DNA was obtained. Such experiments indicate that there must be a specific kind of DNA in each type of pneumococcus.

Transmission of hereditary traits in Diplococcus pneumoniae by DNA is an excellent example of one of the fundamental principles of heredity. It shows that the DNA of the chromosomes of pneumococci affects the cells in which it is introduced to make a particular type of capsular gum.

There were other evidences put forth as early as 1950 in support of DNA as the hereditary material. Some of them are given below.

1. The amount of DNA is remarkably constant from cell to cell, even in different tissues of the body, whereas those of RNA and protein are variable. Since the sperm and egg are haploid, they contain half the amount of DNA, and in polyploids, it is correspondingly more. This exact correlation with polyploidy emphasises the genetic importance of DNA.

2. The wavelengths of ultra-violet rays, which are strongly mutagenic, are found to be those which are highly absorbed by the nucleic acid. Analogues of nucleic acid components and chemicals which react with them are also mutagenic.

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3. The experiments of Hershey and Chase prove convincingly that DNA and not protein is the hereditary material. The shell of the bacteriophage which infects Escherichia coli is of protein while the inner content is DNA. Hershey and Chase made the phosphorus of DNA (32p) and the sulphur of protein of the shell (35S) radioactive. A DNA molecule does not possess sulphur. It is possible to distinguish between radioactivity due to phosphorus and that due to sulphur. The ranges of beta decay particles emitting from radioactive phosphorus and sulphur are different. Their half-lives are also different. When the cells of E. coli are infected with bacteriophage, only DNA enters the bacterial cell while the protein shell remains attached to the cell outside.

Hershey and Chase prepared two separate cultures of *E. coli*. One was labelled with <sup>35</sup>S and the other with <sup>32</sup>p. Bacteriophages were allowed to infect these cultures. The phage particles in the labelled cultures multiplied and also became labelled either with <sup>35</sup>S or <sup>32</sup>p. When bacterial cells lysed, labelled phage particles were released. These phage particles were then allowed to infect two separate unlabelled cultures, i.e. the <sup>35</sup>S-labelled phage one culture and the <sup>32</sup>p-labelled phage another culture. It was observed that bacterial cells in the former did not become labelled while in the latter culture they were labelled (Fig. 30.3). The empty shells of bacteriophage were washed from the bacterial cells and the two were separately tested for their radioactivity. Since it was found that <sup>35</sup>S was present in the protein coat which remained outside the bacterial cell and all the radioactive phosphorus in the bacterium, only DNA could have entered the bacterial cell, and the phage particles that were released must have been formed by this DNA. In other words, DNA is the hereditary material.

### RNA in TMV

In tobacco mosaic virus (TMV), RNA is the hereditary material. It is possible to separate the protein and RNA components of different strains of TMV. They can also be combined to form active 'hybrid' viruses by the cross-combine method. In each case, the hybrid virus made in this way when allowed to infect a tobacco plant, produces disease symptoms characteristic of the parent virus which had provided the RNA, irrespective of their differences in the protein coat (Fig. 30.4). The hybrid virus preparations caused the same disease, as long as they had the same RNA. These results clearly indicate that RNA is the chief carrier of genetic information from the parent to the offspring in TMV.

Since 1950, it has become very clear that DNA is the genetic material. Almost all experiments made to find out the chemical nature of the gene added strength to this conclusion just as experiments in linkage have given additional evidence in support of the chromosomal theory of heredity.

It can now be general sed that DNA is the genetic material except in some viruses like TMV where it is RNA. However, it has no DNA at all.

receptordingly more. This of a constitution to be polytropide en placed and let consider

### Structure of DNA

We have now seen that DNA is the hereditary material. As regards its chemical nature, we also know that it is a polynucleotide and composed of pentose sugar decxyribose and four

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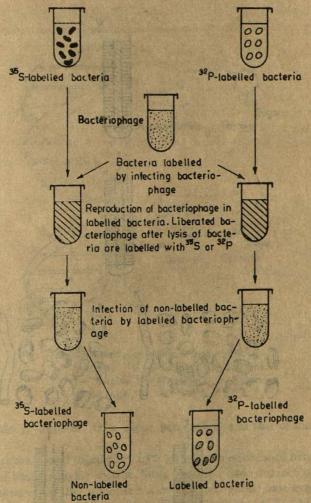


Fig. 30.3 Diagrammatic representation of experiment done by Harshey and Chase in bacteriophage.

nitrogen bases, namely, adenine, guanine, cytosine and thymine, and phosphate molecules. Now the question arises about its structure. Different models of its structure have been suggested, but the one proposed by Watson and Crick is the most accepted one. This model is based on their own work, the work of Chargaff on the relative proportions of four nucleotides in the polymer, i.e. base composition, and the x-ray diffraction patterns studied by Wilkins. This model accounts very well for many of the known physical and chemical properties of DNA and is also capable of accounting for the gene properties.

# Watson and Crick Model of DNA

The essential features of the DNA model (Fig. 30.5) suggested by Watson and Crick are given below.

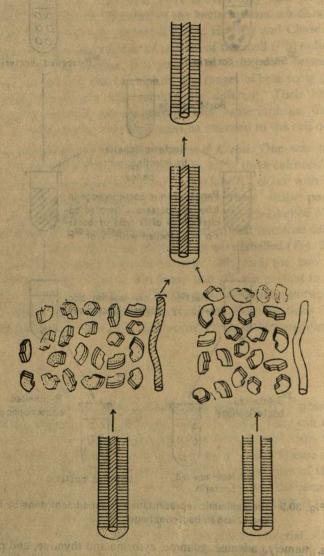


Fig. 30.4 First row- Two strains of TMV (short T.S). Second row- Two strains broken down, Third row- Building of virus with protein of one strain and RNA of the other. Fourth row- The progeny of this hybrid has the protein originally associated with their RNA. (Redrawn after Fraenkel-Conrat, 1956.)

1. Each DNA molecule consists of two long polynucleotide chains or strands which run in opposite directions forming a double helix around a central axis. The diameter of the helix is 20 Å. The strands are made of deoxyribose sugar and phosphate molecules which alternate with each other.

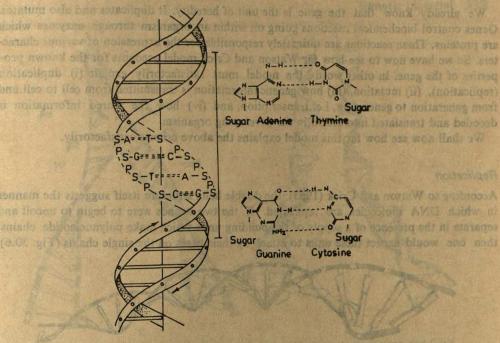


Fig. 30.5 DNA model of Watson and Crick. A-Adenine. G-Guanine, C-Cytosine. T-Thymine. P-Phosphate. S-Deoxyribose sugar.

- 2. Each nucleotide is disposed in a plane perpendicular to that of the polynucleotide strand.
- 3. The two strands are bound together by hydrogen bonds established between base pairs.
- 4. The pairing of bases is highly specific. Since there is a fixed distance of 11Å between the sugar molecules in the opposite nucleotides, one purine base can pair only with one pyrinudine. So the pairs that can be formed are only the A-T and G-C pairs. Two hydrogen bonds link A and T while three hydrogen bonds are required to pair G and C. Because of this arrangement of bonds, A-C and G-T pairs cannot be formed. Hydrogen bonds are weak.
- 5. There may be considerable variation in the axial sequence of bases along one polynucleotide chain but on the other strand, the sequence must be complementary as indicated below:

First strand Second strand GCTCGTGTCACG CGAGCACAGTGC

Because of this property, if a sequence of base on one strand is given, the sequence on the other strand becomes known as it is complementary.

6. A complete turn of the strand (pitch) takes place every 34 Å, and 10 nucleotide units are present in this length.

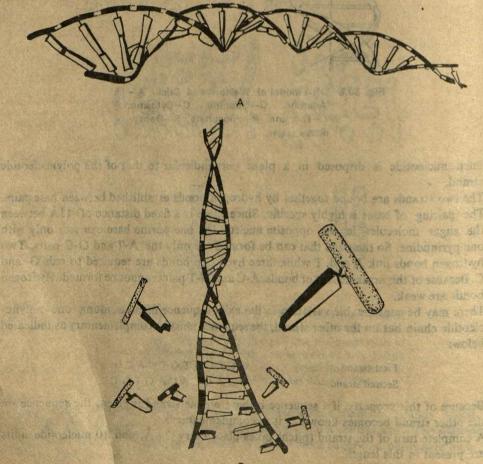
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We already know that the gene is the unit of heredity. It duplicates and also mutates. Genes control biochemical reactions going on within an organism through enzymes which are proteins. These reactions are ultimately responsible for the expression of various characters. So we have now to see how far Watson and Crick model accounts for the known properties of the gene. In other words, the model must satisfactorily explain (i) duplication (replication), (ii) mutation, (iii) how genetic information is transmitted from cell to cell and from generation to generation, i.e. transcription, and (iv) how the stored information is decoded and translated into action in the developing organism.

We shall now see how far this model explains the above criteria satisfactorily.

### Replication

According to Watson and Crick (1953), the double helix structure itself suggests the manner in which DNA molecules are replicated. If the two strands were to begin to uncoil and separate in the presence of a supply of the building units which make polynucleotide chains, then one would expect these units to attach by their bases on the single chains (Fig. 30.6).



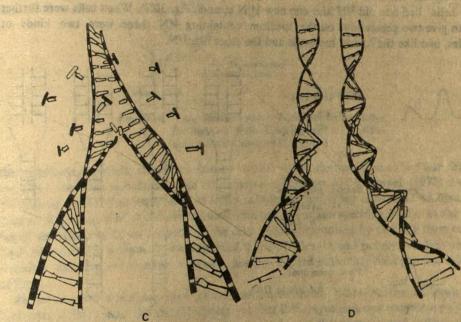


Fig. 30.6 Explanation of idea about replication of DNA molecule by Watson and Crick.

A—The helix carrying coded rungs is ready to divide. B—Two helixes start to grow from splitting the original. C—As the helix unzips, new code units converge. D—Replication process is complete and two helixes identical to the original helix are formed.

In each case, attachment by hydrogen bonding would only be possible for the correct complementary set of nucleotide units which would progressively be built up by base pairing on each side of the two original strands acting as templates. Finally there would be linking up of the complementary sets of units to form DNA molecules, each of them precisely a copy of the original molecule. This type of DNA synthesis is called semi-conservation replication because each of the daughter DNA molecule is composed of one old strand of the parent molecule and one newly synthesised strand.

Experiments have shown that the DNA molecule unwinds so that each strand may serve as a template for a new complementary strand. Meselson and Stahl performed an ingenious experiment in 1958 which convincingly demonstrated the semi-conservative nature of the

The technique involved in the experiment of Meselson and Stahl is of density gradient in a centrifuge tube. A solution of caesium chloride kept in a high centrifuge tube sets up a gradient of concentration depending upon the equilibrium between the centrifugal force and the motion of molecules. DNAs of different density within the continuous gradient will come to rest at different distances from the centre of rotation. Meselson and Stahl grew E. coli bacteria for many generations in a medium containing heavy nitrogen 15N. The cells were then allowed to grow on a medium containing 14N long enough to divide once. At that were then allowed to grow on a medium containing 14N long enough to divide once. It time, the DNA was extracted from the cells and kept in the density gradient centrifuge. It was observed that the equilibrium position was exactly half-way between that of 14N and

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15N proving that the DNA molecules were hybrid in nature as per expectation since each double helix had one old 15N and one new 114N strand (Fig. 30.7). When cells were further grown to give two generations on the medium containing 14N there were two kinds of molecules, one like the hybrid molecule and the other like 14N.

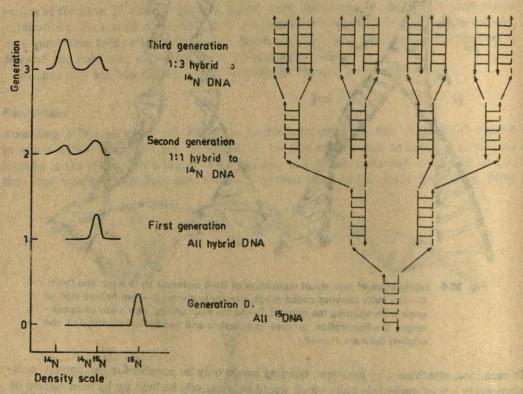


Fig. 30.7 Diagrammatic representation of the results obtained by Meselson and Stahl in connection with DNA replication.

Experiments were also made on higher plants and they also led to more or less similar conclusions. In this connection, the experiment performed by Taylor is a brilliant example. He used thymidine which is thymidylic acid without the phosphate acid group. One of the hydrogen atoms of thymidine is replaced by tritium (<sup>3</sup>H) which is an isotope of hydrogen possessing two additional neutrons. Tritium is radioactive and its autoradiograph can be made when exposed to a particular type of film. Taylor grew root tips of *Vicia faba* in the presence of radioactive thymidine which was incorporated into DNA as deoxythymidilic acid. He observed its distribution throughout all the chromosomes. He then permitted the chromosomes to divide once in the absence of a radioactive label. When the autoradiograph (Fig. 30.8) was examined by him, it was seen that the outline of the chromosome could be traced because of the emission of beta particles. It will be noticed that at some places there is duplication of some parts, e.g. the part on the right side, indicating new radioactive DNA strands. These observations were, of course, at the chromosomal level and not the molecular level. But at the same time, they lead to the conclusion that there is a basic 'doubleness' to

was meaning that the confliction coulder has seedy had any parties of between the

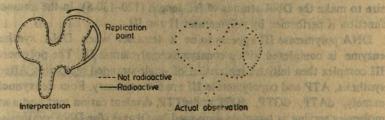


Fig. 30.8 Proof for DNA replication from direct autoradiographs from DNA labelled with radioactive tritium obtained by Taylor.

the replicating genetic structure. It may be as a visible chromosome as in Vicia faba or as the DNA molecule in bacteria. The experiments of Prescott and Kuempel (1972) furnished convincing proof that replication of DNA is bidirectional. They allowed DNA synthesis to start in the presence of thymidine labelled with tritium at low specific activity. At the beginning of the round of DNA replication, the cells were synchronised by starving them of amino acids necessary for growth. It was observed that DNA length was poorly labelled while the stretches flanking on its either side were heavily labelled indicating bidirectional replication of DNA.

Recent researches indicate how DNA replication takes place at the molecular level. Kernberg (1957) was the first to point out that DNA synthesis was enzyme catalysed and the enzyme could be isolated. He succeeded in isolating the enzyme and called it DNA polymerase. It was subsequently observed that the progeny molecules of DNA synthesised by E. coli polymerase were branched molecules although the present molecules were straight chains. Figure 30.9 illustrates a possible mechanism by which branching may occur. Okazaki et al. (1968) observed DNA replication in bits. Kernberg's enzyme is now found to be polymerase I. According to Tait and Smith (1974), DNA polymerase III participates in the synthesis of short pieces of DNA (20 S) from individual nucleotides. The DNA pieces of intermediate size (70-120 S) are synthesised from these short pieces with the aid of

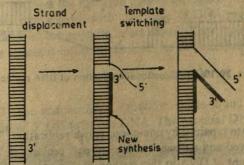


Fig. 30.9 A possible mechanism by which progeny molecules are branched in case of DNA synthesized by *Escherichia coli* polymerase. (After Woodward and Woodward, Concepts of Molecular Genetics, 1978, with permission from McGraw-Hill Book Co.)

polymerase-I. Polymerase III comes into action again and extends the pieces of intermediate size to make the DNA strands of full length (120-130 S). In the absence of polymerase I, its function is performed by polymerases II and III.

DNA polymerase III appears to be a tetramer when DNA synthesis is initiated. This enzyme is complexed with a coenzyme copolymerase III. The polymerase III-copolymerase-III complex then initiates replication of single-stranded templates. After initiation of DNA synthesis, ATP and copolymerase III are not necessary. Four deoxynucleoside triphosphates, namely, dATP, dGTP, dCTP and dTTP, divalent cation mg<sup>+</sup> and a small number of DNA molecules serving as template were used by Okazaki for DNA synthesis. No DNA synthesis was possible in the absence of DNA template. He isolated the enzyme and called it DNA polymerase. The synthesis of a new strand always occurred in the 5' to 3' direction, i.e. two strands are synthesised in opposite polarity.

Because of the formation of bits of DNA, the process of DNA replication is not continuous. Several newly formed fragments grow simultaneously in tandem on both the templates. These fragments are then joined together by the enzyme DNA legase, and thus continuous strands are produced. Recent researches have indicated that for starting DNA synthesis, RNA is necessary. It acts as a primer. The enzyme RNA polymerase synthesises a short RNA (~100 nucleotides) which is complementary to one of the DNA strands. The 3'OH of the terminal ribonucleotide acts as the primer for DNA synthesis by DNA polymerase III.

During DNA replication, two processes—unwinding of the parental strands and synthesis of new strands—occur simultaneously. The junction at which the two processes meet is called the replication fork (Fig. 30.10). At this point, the parental strands are linked with hydrogen bonds in front and separated in back. New DNA molecules are synthesised on both the separated strands up to the fork point. The replication fork during DNA synthesis moves from the point of initiation of synthesis to the point of termination.

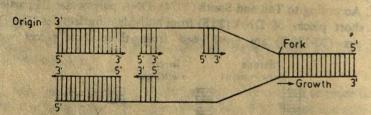
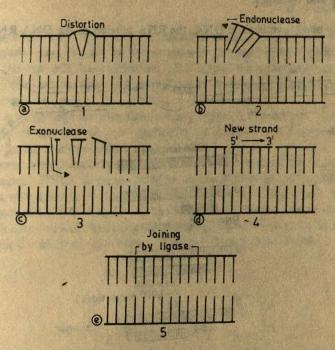


Fig. 30.10 Unidirectional replication of DNA molecule.

### Repair Replication

Damaged nucleotides are repaired by non-conservative replication. The damaged part of the parental DNA is removed and a newly synthesised part replaces it. This means that there is no increase in the amount of DNA in this method of non-conservation. This mechanism has been demonstrated experimentally in case of damaged DNA molecules. X-rays and ultraviolet radiation were used to cause damage to DNA molecules. In this process, enzymes, endonuclease, exonuclease, DNA polymerase and polynucleotide ligase take part. It involves the following steps (Fig. 30.11):



DNA replication for repair. 1. Distortion due to ultra-Fig. 30.11 violet or x-rays. 2. Broken strand due to endonuclease. 3. Excision process through exonuclease. 4. Synthesis of new strand in 5'-3' direction. 5. Joining of the newly formed strand through ligase.

- 1. The enzyme endonuclease (incision enzyme) recognises the damaged or distorted parts of the DNA double helix and it causes breaks in one of the strands near the damaged points.
- 2. Exonuclease enzyme removes the damaged part of the strand.
- 3. New nucleotides are synthesised with the help of DNA polymerase and these are complementary to those of the opposite intact strand. They replace the damaged parts.
- 4. The new part of the strand is linked by the enzyme polynucleotide ligase.

### In Vitro DNA Synthesis

In this process (Fig. 30.12), the endonuclease produces single-strand breaks called nicks resulting in the formation of 3' OH template DNA. The parental DNA is displaced by the DNA repair synthesis of polymerase I. The enzyme ligase joins the broken ends of the parental DNA resulting in the formation of a 4-stranded intermediate. The newly generated DNA fragments are also connected together by ligase. RNA polymerase is used for synthesising a short RNA (approx. 100 nucleotides). The latter is complementary to one of the DNA template strands. The role of the 3' OH of the terminal ribonucleotide is of the primer for the synthesis of new DNA (a fragment of approx. 100 nucleotides) by DNA polymerase III. For getting only newly synthesised DNA, the RNA portion of the DNA-RNA chain is to be hydrolysed.

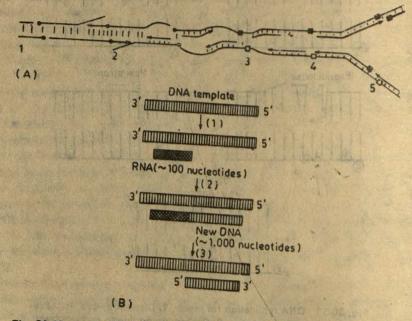


Fig. 30.12 In vitro DNA synthesis. A—Steps in DNA synthesis as follows:

1. Strand breaks at places due to endonuclease action and 3'OH DNA template is formed. 2. Parent DNA is set aside due to synthesis of polymerase I. 3. Broken ends of parent DNA are joined due to ligase. 4. Intermediate of four strands is formed. F. New DNA pieces are formed through ligase. —Nick caused in DNA strand due to endonuclease. —Some sites after rejoining. —Place of progeny DNA. B—Suggested role of RNA in DNA replication as in stages in A. (Redwayn from Woodward and Woodward, Concepts of Molecular Genetics, 1978.)

Sinsheimer observed that the chromosome of small bacteriophage X174 is constituted by only a single-stranded molecule of DNA. It is circular and the smallest chromosome recorded so far. It is about 6,000 nucleotides in length and about 6 to 8 genes are located therein. It is possible to infect cells of *E. coli* with 'raw' DNA. In other words, infection can occur without using whole phage particles. Kornberg had an idea that the bacteriophage X174 would be an excellent experimental material to test the biological activity of DNA. Hence, he used X174 DNA as a template in the DNA synthesis. The newly produced DNA molecules were able to infect cells of *E. coli*.

#### Mutation

The second property of the gene to be explained on the basis of the DNA model suggested by Watson and Crick is mutation. If the structure of DNA is examined, it will at once be clear that since the strands are composed of a monotonous alternate sequence of sugar and

phosphate molecules, they could not be causing mutation. It is the base sequence that is variable. Therefore, any change in one or more of the bases will be reflected when a new complementary strand will be synthesised. The size of the mutational change could be a single base substitution or there the loss of one or more base molecules or there could be more complex arrangements.

Mutations of a single base are classified into two types: (i) Transitions, which are changes from one purine to another or one pyrimidine to another. (ii) Transversions, which are

changes from a purine to a pyrimidine or vice versa.

We should expect that chemicals which affect purines or pyrimidines should act as mutagens. For instance, HNO2 changes cytosine to uracil (Fig. 30.13). Uracil can then form hydrogen bonds with adenine so that the change will be from a C-G pair to a T-A pair through the sequence C-G, U-A and T-A. Similarly, HNO2 changes adenine to hypoxanthine which pairs with cytosine and the change is ultimately from A-T to G-C. So we find that by using HNO<sub>2</sub> both the kinds of transitions A-G and G-T can be produced.

Fig. 30.13 Transformation of cytosine to uracil serving want of a salary due to nitrous acid.

It has already been stated at the end of Ch. 25 that our ideas are undergoing a profound change because of new findings.

The recent work on a transposable element in Streptomyces coelicolor (Sermonti, 1981) indicates that a transposon is flanked by two other shorter DNA segments called insertion sequences (IS) which execute its transposition. These segments function as biological regulators by switching on or off genes or group of genes from the position of their insertion. They also produce deletions of adjacent DNA regions. A transposon carrying a chloramphenical resistance gene has been found in S. coelicolor. It seems to be located adjacent to the transfer gene (s) and arg operon.

Mutations caused by insertion or excision of IS's have been found to be frequent not only in prokaryotes but also in eukaryotes. It has been observed that IS's can also produce genome alteration in specific regions at a very high frequency, thus causing many genetic unstabilities. The effect of a mutagen in inducing operations of IS is, however, unknown.

An IS segment resembles a provirus in many respects with various possible localisations on DNA. The mu (mutator) virus is responsible for producing mutations at a high frequency by inserting in various regions of the bacterial genome. This virus can be regarded as a prototype of an IS segment (Sukhari et al., 1977).

It appears that mutagens like ultraviolet rays, ionising radiations and many alkylating agents induce mutations in a way which can be compared to the way they induce prophages. Their action involves induction of a series of functions called SOS functions. On one side, side as every to economic summer supported the administration and so total to you ago. the enzymes (endonucleases) producing the single-strand excision of the DNA segment are induced by them. The segment bears the mutagen-caused lesion. In the case of ultraviolet rays, it is a thymine dimer. On another side, they produce the depression of a special polymerase which is concerned in patching the excision gap by copying the residual strand. This DNA polymerase is called the *DNA-mutase*, which is inaccurate in copying (error-prone). The erratic behaviour of this polymerase is responsible for causing mutations. There are strains which are constitutive for they do not need mutagen induction. They mutate spontaneously at a high rate.

Another system making the so-called 'post-replication repair' has been detected in the bacterial cell (Hanswalt and Setlow, 1975). It has been found that this system works erratically, i.e. it is error-prone. It functions as the multiplicity-reactivation in phages. In other words, segments are recombined with lesions in the non-homologous sites.

The above mentioned work indicates that the trend in respect of mutagenesis is moving towards a viral model. There are still questions which we are not yet in a position to answer, e.g. why does a cell mutate? Why is the repair system error-prone? It is, however, clear that mutation is a function of the cell.

### Storage of Information

This is found in the sequence of the purine and pyrimidine bases. A single gene probably carries considerable information. For example, one gene (cistron) possesses about 1000 to 1500 nucleotides. So the number of permutations is 41000 (since there are 4 bases) or 41500. This number is much more than the number of elementary particles in the known universe. When we consider the number of genes present in a cell, we can well imagine the enormous amount of information contained in the base sequence in DNA.

When one of the strands of a double helix is specified, the other is known because of its complementary nature. Hence, opposite each A there must be T and opposite each G a C. This aspect will be dealt with later on.

### Translating Genetic Information into Developmental Activity

In this connection, we should realise that the information encoded in the DNA is used to specify a particular enzyme, i.e. protein, because genes exert their influence through enzymes in the control of biochemical reactions which are responsible for the development of an organism. Proteins are complex three-dimensional molecules. Their structure is determined by the sequence of amino acids in the linear polypeptide. When protein is denatured, its three-dimensional structure is broken down into one-dimensional threads and its biological activity is destroyed. This process is usually reversible in vitro. When the thread comes back into its original three-dimensional structure, its biological properties (e.g., acting as an enzyme) are again restored. These experiments indicate that the full information for the structure is stored in the primary sequence of amino acids. The primary structure, which is linear, determines the three-dimensional structure (tertiary). Recent techniques have now made it possible to determine the amino acid sequence in many proteins.

Some amino acids in proteins are also present in a linear sequence. It seems that there might be a decoding system which enables to determine a particular amino acid when a sequence of three or four nucleotides is known. Therefore, a long sequence of bases in the

nucleic acid should be capable of dictating a specific arrangement of amino acids in protein. For instance, the sequence GGA might indicate proline, TGA threonine, CTC glutamic acid, and so on. In other words, each three-lettered word spelling a different amino acid. So the nucleic acid sequence GGA, TGA, CTC should lead to the formation of a protein with the amino acid sequence.

### proline → threonine → glutamic acid

What we have considered in the above paragraph is corrobor ted by the remarkable study of haemoglobins in several hereditary anaemias by Ingram. He demonstrated that three haemoglobins due to three alleles at the same locus differ in only a single amino acid substitution. Let us assume that there is an abnormal protein derived from the mutation of a gene. When a chemical analysis of this abnormal form and a normal one is done, we will be able to find out how they differ. If we succeed in this, we may know about the site of the gene action and magnitude of the chemical change. Ingram's work was on this line. He has shown that haemoglobins A, S and C (arbitrarily designated) have the following amino acid sequences in one part of the molecule while they are identical in the rest of the molecule (Table 30.1).

| Table 30.1 | Sequence of | amino acids | in haemoglobins |
|------------|-------------|-------------|-----------------|
|            |             |             |                 |

| Amino acid  | Haemoglobin   | Haemoglobin  | Haemoglobin  |  |
|---|---|--|--|--|
| number  | A   | S  | C  |  |
| 1 1 2 2 1 8 1 2 1 8 1 1 2 1 8 1 1 2 1 1 1 1 | Histidine Valine Leucine Leucine Threonine Proline Glutamic acid Glutamic acid Lysine | Histidine Valine Leucine Leucine Threonine Proline Valine/ Lysine Glutamic acid Lysine | Histidine Valine Leucine Leucine Threonine Proline Valine/ Lysine Glutamic acid Lysine |  |

A haemoglobin molecule consists of four parts—two alpha chains and two beta chains of amino acids. The chemical changes which we have just considered are found in the beta part while haemoglobins, e.g. G and I are caused by amino acid substitutions in the alpha chain. These mutants are at a different gene locus from that which causes A, S and C mutants. This is in accordance with the idea that the alpha and beta chains are formed because of the action of different genes which are present either on different chromosomes or are wide apart on the same chromosome to allow free recombinations.

From the above, it appears that the order of the bases in DNA could be determined and this compared with the other of the amino acids in the polypeptide formed by the gene. This would reveal the system of coding. However, so far, it has not become possible to determine the base sequence of DNA of any great length though considerable information on protein and amino acid order is available and one can compare this with genetic information.

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In E. coli, Yanofsky identified a series of closely linked mutants affecting the enzyme tryptophan synthetase. He was able to find out what the amino acid replacement was and the location of this amino acid in the polypeptide. He noted that mutant sites were not only in the right sequence but the distances were also approximately proportional to the number of amino acids separating them. This work clearly indicates that there is a linear correspondence between the nucleotide sequence in the gene and the amino acid sequence in the protein.

### Structure of RNA

The primary structure of RNA is similar to that of DNA except that ribose sugar and uracilare present instead of deoxyribose sugar and thymine. The molecules may be single- or two-stranded like DNA. Three types of RNA have been identified on the basis of molecular weight and other properties. These are ribosomal RNA (rRNA), messenger RNA (mRNA) and soluble or transfer RNA (tRNA). These are derived from the nucleus and take part in the synthesis of enzymes and other proteins.

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### One-gene, One-polypeptide Chain

We have already seen that Beadle and Tatum proposed the one-gene, one-enzyme theory. This has now been modified to the one-gene, one-polypeptide chain in the light of recent researches. It has been seen that some enzyme activities are because of the specific association of different kinds of polypeptide chains while others result from single polypeptide chains. Besides, a single protein may catalyse two different metabolic reactions. Recent information on enzymology and protein structure as well as refined genetic techniques have permitted the more precise correlation of the gene and polypeptide chain rather than the gene and enzyme chain. The gene-polypeptide relationship is simple and also has far-reaching significance. In fact, its validation should be regarded as one of the triumphs of modern genetics. The polynucleotide as well as the polypeptide are not only linear structures but also colinear.

# Genetic Code

When the linear nature of DNA and polypeptide chains was recognised, speculations started about the nature of the correspondence between the nucleotide and the amino acid order. We have already seen that the four bases of the DNA molecule form a four-letter genetic alphabet. Now, the problem is about the number of letters 1, 2, 3 or 4 forming a code for a specific acid. In other words, what is the size of the coding unit?

We have gained considerable knowledge about the genetic code owing to the brilliant researches of Ochoa, Nirenberg, Khorana and others. The studies of the mutagenic effects of acridine have also aided in our knowledge of the coding unit. Proflavin acts as a powerful mutagen for some bacterial viruses when it is present during DNA replication. The mutations induced by proflavin give rise to one base duplication or deletion. Sometimes, a

combination of three deletion mutations very close together, or three duplications will lead to the formation of a normal or nearly normal phenotype. These experiments show that the coding unit consists of a triplet code consisting of three letters of three bases. This unit is known as a codon.

The interpretation of Crick and his associates in this connection is as follows. Protein synthesis begins at one end of the messenger RNA and goes on along it, translating three bases at a time into protein. If a base is added or deleted by mutation, from that point the 'reading frame' is so shifted that the rest of the message is misread. It is suggested that the code is a three-letter (or otherwise a mutation of three) code and that the translation mechanism 'reads' triplets by threes.

Nirenberg in one of his earlier experiments, observed that an RNA composed only, of uracil acted as a messenger and caused the synthesis of a polypeptide containing only phenylalanine. So the codon for phenyl alanine must be UUU and the corresponding DNA is AAA. It is customary to give codes in terms of sequence of mRNA.

The work of Ochoa and Nirenberg has added much to our knowledge concerning genetic code. For instance, poly-C produced a polypeptide composed only of proline and poly-A formed lysine.

In 1964, Nirenberg observed that tRNA was attached specially to the ribosome-mRNA complex under certain circumstances. This attachment was found to be specific. Only lysine RNA was attached to AAA AAA messenger RNA. Very short messenger RNAs comprising three units caused a specific tRNA to attach. We now know most of the codons with certainty (Table 30.2).

Table 30.2 Genetic code

| First  | Second letter |            |  |      |        |
|--|---------------|------------|--|------|--------|
|  | U             | C          | A man  | G    | letter |
| C) 25 1050 5   | Phe           | Ser        | Tyr  | Cys  | U      |
| Phe U Leu Leu Leu  |               | Ser        | Туг  | Cys  | C      |
|  |               | Ser T      | Ochre  | Opal | A      |
|  | Ser           | Amber      | Try  | G    |        |
|  |               | Pro        | His  | Arg  | U      |
| C Leu<br>Leu<br>Leu  |               | Pro        | His  | Arg  | C      |
|  |               | Pro        | Gln  | Arg  | A      |
|  |               | Pro        | Gln  | Arg  | G      |
|  |               | Thr        | Asn  | Ser  | U      |
| Ile Ile A Met Met Val Val  |               | Thr        | Asn  | Ser  | C      |
|  |               | Thr        | Lys  | Arg  | A      |
|  |               | Thr        | Lys  | Arg  | G      |
|  |               |            | Asp  | Gly  | U      |
|  | Val           | Ala        | Asp  | Gly  | UCA    |
|  | Val           | Ala        | COLUMN TRANSPORT DESCRIPTION OF THE PARTY OF | Gly  | A      |
| A STATE OF THE PARTY OF THE PAR | Val<br>Val    | Ala<br>Ala | Glu  | Gly  | G      |

Khorana and his associates adopted a different approach. They succeeded in synthesising DNA in which there were repeating triplets with a total length of about 12, e.g. TTC, TTC, TTC. They also prepared repeating doublets of about the same length, e.g. AG AG AG AG AG AG AG. The synthetic RNA prepared from this DNA was found to be much longer and could be used as a messenger for synthesis in vitro. So it was possible to make very specific tests for codes. For example, it was found that the sequence AGAGAG produced a polypeptide consisting of glutamic acid and arginine in alternate sequence. This is in agreement with the codes AGA for arginine and GAG for glutamic acid. These findings also furnish proof in support of the triplet code.

The genetic code is non-overlapping. It is commaless, i.e. continuous. It is also degenerate, i.e. there are several ways of making the same amino acid. For instance, GGU, GGC, GGA, and GGG all code for glycine, Sometimes, the code gives what are called 'non-sense' mutations. For example, UAA and UAG do not code any amino acid. However, they appear to terminate protein synthesis at any point and release a short polypeptide into cytoplasm.

# Genetic Control of Protein Synthesis

There is still a large gap in the information about DNA-specified protein synthesis. DNA is a part of chromosomes which are situated in the nucleus. So it is in the nucleus except in plastids and mitochondria wherein it occurs in small quantities. But actually protein synthesis takes place in the absence of DNA in the cytoplasm. It is associated with ribosomes. So the question arises how DNA in the nucleus controls protein synthesis taking place in the cytoplasm. It is found that the intermediary in this process is RNA.

### Messenger RNA and Ribosomes

Messenger RNA carries the information from the nucleus to the ribosomes situated in the cytoplasm. There is direct proof that mRNA moves from the nucleus to the cytoplasm. Radioactive labelling experiments have been done in this respect. Soon after RNA synthesis, during which radioactive bases are incorporated into RNA, the radioactivity is in the nucleus and later it is observed in the cytoplasm.

If mRNA acts as the messenger, then we have to distinguish two processes:

- 1. Transcription of the information from DNA to mRNA.
- 2. Translation of RNA information into protein.

A number of investigators are involved in finding out how the message with specific instructions about making a particular protein and in what quality is sent by DNA to the ribosomes. At first, the above mentioned two processes appeared to seem unrelated. One group of investigators was making an attempt to identify the enzymatic machinery employed by the cell to synthesise RNA from nitrogen bases and other building blocks while the second group was trying to understand how the DNA of a virus like T<sub>2</sub> seizes upon the metabolism of the host cell and converts it to the virus.

DNA is thought either to act as a master template or die in the manufacture of proteins. The templates are stored in the nucleus as bundles of double complementary strands. The first controlled RNA synthesis was done by Ochoa in 1956 by using the enzyme polynucleotide phosphorylase.

Transcription takes place when mRNA is synthesised in the nucleus. It is made with the help of an enzyme RNA polymerase. This enzyme works only when DNA is present. The mRNA should be complementary to the DNA which serves as its template (Fig. 30.14). There is ready formation of 'hybrid' DNA-RNA molecules and this fact proves that the mRNA is complementary to the DNA strand.

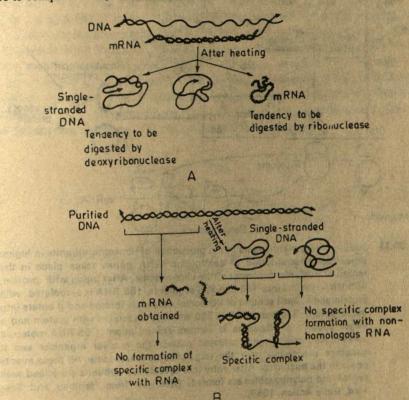


Fig. 30.14 A-Part of DNA molecule along which mRNA is synthesized. 8-specific mRNA and RNA-DNA complex formation in the single-stranded DNA in the same gene (Redrawn from Hartman and Suskind, Gene Action, 1969).

Only one of the two DNA strands is involved in the copying process which is expected. If it were not, each gene would prepare two kinds of polypeptides. There is, of course, no proof of this. How the choice is made is not, however, known. mRNA after it moves from the nucleus to the cytoplasm, becomes associated with ribosomes.

Ribosomes are relatively non-specific work benches from which tRNA molecules move to and fro like a shuttle carrying amino acids required for the synthesis of polypeptide chains (Fig. 30.19). The specific sequence in which the charged tRNA molecules are used depends

We have already seen that in bacteria, ribosomes consist of two subunits, 30S and 50S, on the code contained in mRNA molecules. and that they are synthesised in the nucleolus. After their entry into the cytoplasm, the subunits attach themselves to the mRNA (Fig. 30.15), and the formation of polyribosomes starts. Certain proteins which are a part of the newly emerging subunits as well as rRNA are shed during the formation of polyribosomes.

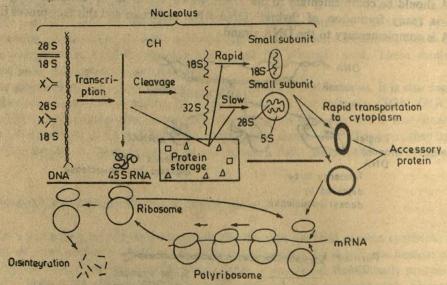


Fig. 30.15 Outline of important events in the production of ribosome subunits in higher organisms. Transcription of contiguous rRNA genes takes place in the region of nucleolar organizer of the chromosome. After union with protein, 45S RNA cleaves into 18S and 32S segments. 18S RNA is associated with protein and a small subunit of ribosomal subunit is formed and it enters into cytoplasm. 3 S is changed into 28S which is associated with protein and a larger subunit of ribosome is formed. Transcription of 5S RNA molecule takes place through genes situated outside the nuclear organizer and it becomes associated with the large subunit of ribosome. All these events occur in the nucleolus. After entry into cytoplasm, subunits are joined with mRNA and polyribosomes are formed. (Redrawn from Hartman and Suskind, Gene Action, 1969).

Free 70S ribosomes are not the units very highly active in normal cells. The most active units are actually aggregates consisting of an mRNA molecule to which a number of 70S ribosomes are bound. The ribosomes seem to be spaced along the mRNA molecule in such a way as though they were translating its different portions. There is proof that polyribosomes are active in protein synthesis. There is incorporation of amino acids into proteins in cell-free and in vivo systems.

In the case of eukaryoteic cells, not only are the rRNA molecules larger (18S and 28S) but also the ribosome particles (40S and 60S) which carry them. A complete ribosome is 80S. The transcription of 18S and 28S RNA molecules takes place as a single RNA molecule with the weight of a ribosome particle about 45A. The 45S unit is cleaved after synthesis into 18S and 28S. Then methylation of many bases occurs in 18S and 28S RNA molecules. It has been observed that genes responsible for 18S and 28S RNA are aggregated in the region of the nucleolar organiser of one or more chromosomes.

Proper binding between mRNA and ribosomes is a necessary step in the translation of the message into the amino acid order of a polypeptide chain. It makes certain initiation of a polypeptide chain at the specific positions on the mRNA.

It seems that in bacteria, free 30S ribosomal component, a protein factor and a special 'initiating' charged tRNA molecule form a complex with mRNA under normal living conditions. This complex then unites with a 50S ribosomal component to form a 70S ribosome active in peptide bond formation. When this active 70S ribosome moves towards the 3' end of mRNA and 5' end gets exposed, there takes place attachment of another 30S subunit at the initiation site on the mRNA. In this way, this process is repeated resulting in the polyribosome formation (Fig. 30.16). It has been seen that the formyl methionine base is at the beginning of a polypeptide chain and the formyl group is afterwards removed by the enzyme deformylase resulting in leaving off methionine behind.

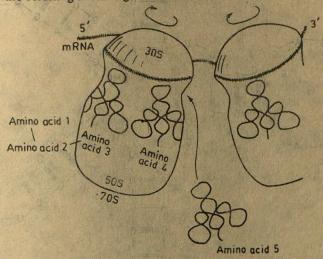


Fig. 30.16 Part of polyribosome active in protein synthesis. As the message in mRNA is successively read, 70S ribosome subunits rotate. (Redrawn from Hartman and Suskind, Gene Action, 1969.)

The shape of tRNA is like that of a clover leaf (Fig. 30.17). It is partly determined by regions of hydrogen bonds between complementary segments of linear single-stranded molecules. These segments of the molecule which do not pair form loops. The recognition sites have been identified within the regions of these loops. Loop 1 is thought to be the recognition site of amino-acyl synthetases. In Fig. 30.17, loop 2 indicates the anticodon which recognises a codon of the mRNA, while loop 4 is considered to be the site of ribosome attachment.

The first step in protein synthesis is amino acid activation. There is a reaction of an amino acid with adinosine triphosphate (ATP) catalysed by a specific activating enzyme, an amino

Fig. 30.17 Transfer RNA molecule of leaf clover shape. Loops 1, 2, 3 and 4 are identification sites.

acyl tRNA synthetase. The first formed product is an amino acyl adenylate which is bound with energy required for the second step involving attachment of the amino acid to a specific tRNA molecule. The adenylate remains attached to the enzyme till it is transferred to the tRNA. This transfer is catalysed by the same enzyme. These reactions are shown in Fig. 30.18.

The synthetases are highly specific under physiological conditions. This means that each of them generally activates only one form of amino acid. Amino acid activation is a reversible reaction.

Fig. 30.18 Activation of an amino acid and its transference to tRNA. R-Amino acid side chain.

The activated amino acids remain attached to the enzymes till their transfer to specific tRNA molecules. The amino acid-tRNA molecules then are attached to ribosomes actively involved in protein synthesis and are specifically oriented in the proper positions on the template mRNA (Fig. 30.19). The mRNA which is synthesised along one strand of DNA molecule becomes attached to the ribosome in such a way that it becomes accessible to the amino-acid-tRNA molecules.

Ribosomes have two sites for binding aminoacyl-tRNA molecules. One is a peptide site and the other is an amino acid site (A site).

In bacteria, it has been observed that before the first amino acyl-tRNA complex (formylmethionyl-tRNA) attaches to the mRNA-30S ribosome complex, mRNA has to have its binding to the 30S ribosome. In this first step, AUG codon of mRNA, 30S ribosome and the initiation factor IF-3 are involved. Little is known about IF-3. The total complex is IF-3-30S ribo-mRNA. The formyl-methionyl-tRNA (tRNAfmet) becomes bound with this complex, for accomplishing which, the initiating factor IF-2 is necessary. The complex II formed is IF-2-30S ribo-mRNA-tRNAf met-GTP. The next step involves attachment of 50S ribosome to this complex and the cleavage of GTP to GDP and the complex III takes place (70S ribo-mRNAtRNAfmet) On the complex, the attachment of tRNAfmet to the P site on 50S subunit of the ribosome is effected. Complex III is the protein synthesising apparatus. When it is formed, elongation of the polypeptide chain starts immediately. The second and rest of the tRNAs, however, attach to the A site. When the amino acid at the A site becomes peptide bonded to the amino acid at the P site, there is movement of the aminoacyl-tRNA from the A site to the P site and the peptide bond is formed. The result of this is that tRNA becomes first dissociated from its amino acid and is then removed from the complex. While there is occurrence of the event, a third aminoacyl-tRNA gets bound to the A site and the process is continued until the translation of the mRNA molecules is completely achieved. In this way, synthesis of a polypeptide chain takes place. Dintzis (1961) indicated that the direction of protein synthesis is from the M' (amino terminal) to the G' (carboxyl terminal) terminal.

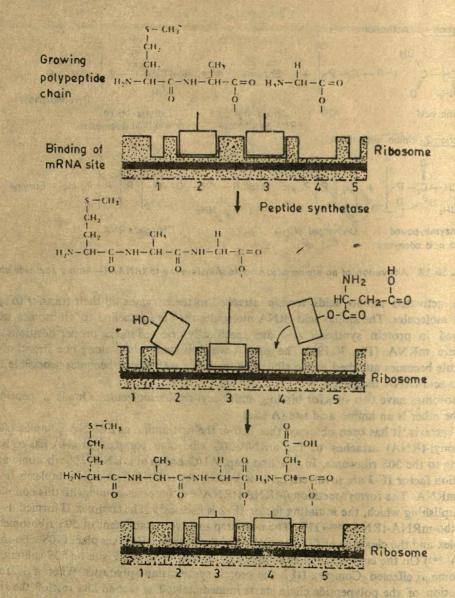


Fig. 30.19 Some steps in growth of a polypeptide chain. Binding sites on the ribosome permit orientation of changed tRNA molecules and mRNA. Though there are many binding sites for tRNA, only two are active at one time on each ribosome. One of the tRNA molecules is responsible for maintenance of the nascent polypeptide chain bound to the ribosome. The second tRNA (top) enters a site adjacent to the one containing the polypeptide chain. A new, charged tRNA which is specific for the mRNA at the site 4 enters this activated site (middle) ready for repeating the sequence of events (bottom). (Redrawn from Hartman and Suskind, Gene Action, 1969).

It has been seen that AUG is the initiation codon of mRNA molecules. When the second aminoacyl-tRNA becomes attached to the A site on the 50S ribosome of complex III, the elongation factor EF-T and GTP are essential.

Termination of the polypeptide chain is effected by one or more codons, UAA, UGA or UAG. They are, therefore, called stop triplets. It appears that at least two termination codons take part in this process. The factor R recognises UGA and UAG and R2 UGA.

It has been observed that there are present certain factors which are required in the process of transcription (Fig. 30.20). The factor sigma (o) is essential for the identification by RNA polymerase of initiation sites on the DNA template. The factor is inactive when it is isolated from the polymerase complex. CAMP has also been noted to initiate and/or continuation of transcription. The factor omega (w) has been found in some preparations of E. coli polymers. It is suggested that this factor is essential to polypeptide elongation. The factor rho (P) is possibly engaged in the termination of the chain. It appears that the factor kappa (κ) may also be involved in the chain termination.

The above account clearly indicates that there is a specific organisation and alignment of amino acids in the chain directed by the sequence of bases in the mRNA which selects the correct complementary base sequence from the charged tRNA molecule pool. It seems that the polypeptide chain folds into the final biologically active configuration while it is still bound to the ribosome. When it is to be released from the ribosome, it is hydrolysed enzymatically from the last tRNA molecule to which it was attached.

#### **Reverse Transcription**

As all viruses multiply in the host cells, all viral RNA must be synthesised within the host cells. It has been seen in the case of many viruses that the process of RNA replication on the whole resembles DNA replication of DNA viruses. The genetic information in these viruses is stored in RNA. Hence, new RNA is synthesised from the RNA template. There exists a group of viruses in which the RNA chromosome is replicated only after the synthesis of a DNA intermediate. After formation of this intermediate, the usual order of events from DNA -> RNA -> protein occurs. In this instance, at first the information from an RNA chromosome is transcribed to DNA transcripts. In other words, there is a complete reversal of information flow, i.e. there is reverse transcription. Temin and Mizutani (1970) and Baltimore (1971) found reverse transcription in some tumour viruses. This discovery showed the possibility of making genes in vitro from RNA transcripts. For this purpose, RNA from any source can be utilised along with the substrates necessary for DNA synthesis. The enzyme acting in this process is reverse transcriptase. This enzyme recognises RNA as template and synthesises DNA. This synthesised gene can then be employed as a template in the case of RNA synthesis and the process is catalysed by the enzyme RNA polymerase.

It has been found that oncogenic viruses (causing leukaemias and sarcomas in many mammals) initiate formation of DNA intermediates. Their chromosome is a single-stranded DNA. It carries a gene which codes for reverse transcriptase known as RNA-dependent DNA polymerase. This was first discovered by Temin and Baltimore. The following reactions occur in three stages during the synthesis of this enzyme:

RNA (+) 
$$\rightarrow \frac{\text{CNA}}{\text{DNA}}$$
  $\stackrel{\text{(1)}}{\text{DNA}}$   $\stackrel{\text{(2)}}{\text{DNA}}$   $\stackrel{\text{(3)}}{\text{DNA}}$  transcription

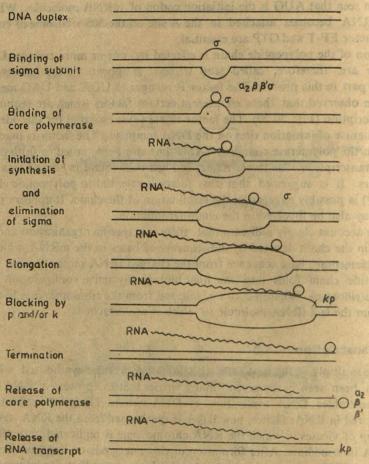


Fig. 30.20 Factors influencing transcription. Steps that can be identified in transcription: (1) Binding of RNA polymerase with DNA. (2) Beginning of growth of chain. (3) Chain elongation. (4) Termination of chain growth and release of RNA polymerase. Factors: Sigma (σ) is necessary for identifying initiation point by polymerase. When sigma subunit separates from polymerase complex, it becomes inactive. Omega (ω) factor found somewhere in Escherichia coil is thought to be necessary for chain elongation. Rho (ρ) role is not yet known, it may be influencing chain termination. The role of kappa (κ) also is not known. (After Woodward and Woodward, Molecular Concepts of Genetics, 1978 with permission from McGraw-Hill Book Co.)

The first stage is catalysed by the RNA-dependent DNA polymerase. In the second stage, the involved enzyme is DNA-dependent polymerase while in the third stage, the catalytic agent is DNA-dependent RNA polymerase. The reverse transcriptase is now used in making genes in vitro.

## Repetitive DNA

Increase in DNA content in modern species by methods like euploidy, aneuploidy and gene duplication takes place. So, the more modern species possess larger genomes than the more primitive species. As regards viruses and bacteria, the size of the genome appears to be determined by the number of vital genes. In the case of mammalian DNA, it is estimated that there are about  $3 \times 10^9$  base pairs per genome. These can manufacture about  $3 \times 10^6$ genes. However, the number of vital genes cannot exceed one-fourth of this number. In other words, more complex species during evolution acquired larger genomes than can be accounted for by the number of vital genes. Hence, a question may be asked about the functioning of the excessive DNA.

Recently, a new kind of DNA was discovered. This new DNA does not participate in the flow of genetic information. It is highly repetitious. Its repeated base sequences are short when compared with structural genes. A higher density DNA has been isolated from the nucleolar organising regions. It represents only a fraction of the total repetitive DNA in all species.

There are many kinds of repetitive sequences of DNA and they differ from species to species. They are aligned along the entire length of the chromosome in tandem. However, we do not know about the function of repetitive DNA. It is thought that it supplies raw DNA from which new functions could be produced. This means that each genome possesses a reserve of DNA in anticipation of new genes and new enzymes. It is also suggested that the repetitive DNA may take part in the pairing of DNA. The presence of repetitive DNA within heterochromatic regions of chromosomes is regarded as a proof of the functioning of this DNA in the pairing of homologous chromosomes.

Honness observed that two plasmids from Drosophila having middle repetitive DNA hybridised at several sites on the Drosophila chromosomes. So he thought that the middle repetitive DNA might be having a regulatory function.

## Left-handed DNA

Recently, a flutter was created among molecular biologists when a report appeared about the experiments conducted by a team of five researchers headed by Alexander Rich at the Massachussets Institute of Technology, USA. The team discovered a DNA molecule with a left-hand spiral. Its twists are jagged rather than smooth, as in the right-handed DNA molecule. The significance of this left-handed DNA is not yet known. Many workers feel that the left-handed DNA may facilitate our understanding of the functioning of the genetic code better. Rich thinks that it may yield new information about the development of cancer. Initial research indicates that the left-handed DNA which Rich cells Z-DNA, may be the catalyser which induces cancer-causing agents to produce malignancy. He has also proposed that the normal right-handed DNA double helix may be turned inside out so as to make the lefthanded pattern by the action of certain chemicals. If this turns out true, the new discovery may mark the first step towards a correct understanding of the causes of cancer and its effective cure in the near future.

Recent researches indicate that what are called split genes are a method of accommodating more DNA than is essential for the functioning of the gene. Prokaryotes have to devise ways

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and means for maximum coding in minimum amount of DNA while in the higher organisms, it appears that there is an excess of DNA. It seems that in these organisms a gene is not contiguous. There are even five or six or more silent regions in a single gene. These regions never appear in the final product. In other words, genes are split. The silent regions are termed inserts or introns.

# SUMMARY

- 1. There is overwhelming evidence that chromosomes carry genetic material. In 1907, Miescher had analysed nucleoproteins from pus cells. Chromosomes have been analysed. They are composed mainly of proteins, DNA and RNA.
- 2. A study of the pestilent bacterium Diplococcus pneumoniae by Griffith led ultimately to the understanding of the chemical nature of genetic material. His experiments on mice with virulent and non-virulent strains indicated that S-cells of the bacterium caused transformation of R-cells into S-cells which are virulent. The extract of R-cells responsible for transformation was called transforming principle. The nature of this principle was brought to light by Avery, McCleod and McCarty in 1941. They showed that it was DNA. Hence, it was proved that DNA is the hereditary material. There is also much other evidence to indicate DNA as the hereditary material. The experiments of Hershey and Chase proved convincingly that DNA, and not protein, is the hereditary material. However, in TMV, RNA is the hereditary material since DNA is absent.
- 3. Watson and Crick suggested a model for the structure of the DNA molecule, which has been accepted. The molecule consists of two long polynucleotide chains or strands running in opposite directions and forming a double helix around a central axis. The strands are made of deoxyribose sugar and phosphate molecules which alternate with each other. These strands are bound together by hydrogen bonds established between base pairs. The pairs that can be formed are only A-T (adenine-thymine) and G-C (guanine-cytosine) pairs. Adenine and guanine are purines while thymine and cytosine are pyrimidines.
- 4. The model explains replication, which is of semi-conservation nature, and mutation very well. This is supported by the experimental evidence. Much information is now available on the process of DNA replication and repair replication. DNA synthesis has been accomplished in vitro.
- 5. The storage of information has been found to be in the sequence of purine and pyrimidine bases. The four bases A, T, C and G, in fact, constitute the four-letter alphabet of genetics. Much work has been done on translation of genetic information into developmental activity. The information encoded in the DNA is used to specify a particular enzyme, i.e. protein, because genes exert their influence through enzymes in the control of biochemical reactions which are responsible for development of an organism. Proteins are made of amino acids. They are polypeptides.
- 6. The seat of protein synthesis is in cytoplasm where ribosomes are present. RNA has an important role in this synthesis. There are three types of RNA, namely, ribosomal RNA, transfer RNA and messenger RNA. These are formed in the nucleus.

8. As regards the genetic control of protein synthesis, there is still a large gap in the information about DNA-specified protein synthesis. Messenger RNA carries the information from the nucleus to the ribosomes situated in cytoplasm. There is transcription of the information from DNA to messenger RNA. DNA is thought to be acting as a master template in the manufacture of proteins. Ribosomes are relatively non-specific work benches from which transfer RNA molecules move to and fro, like shuttles, carrying amino acids required for the synthesis of polypeptide chains. The specific sequence in which the charged transfer RNA molecules are used depends on the code contained in messenger RNA molecules.

9. Reverse transcription has been observed in certain viruses (e.g. oncogenic viruses). They initiate formation of the DNA intermediate. Their chromosome is single-stranded DNA.

10. Recently, a new kind of DNA has been discovered. It does not participate in the flow of genetic information. It is highly repetitious and so is called repetitive DNA. Very recently, left-handed DNA (Z-DNA) has been found. It is thought that this DNA may facilitate our understanding of the functioning of the genetic code better.

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# 31. Nature of Gene

Mendel referred to the hereditary unit as the factor which was called gene by Jansen around the turn of the century. It became known from about 1913 that genes are arranged in a linear fashion like beads in a string along the entire length of a chromosome. Morgan, on the basis of his chromosome theory, brought a distinct definiteness in the gene concept which has now become classical.

#### The Classical Gene

It is possible to study the gene with standard genetic techniques by observing recombination phenotype and mutation. The classical concept of gene envisages it as a unitary particle with the following attributes:

- 1. A gene is a unit of chromosomal structure not subdivisible by chromosomal breakage or crossing over.
- 2. A gene is a unit of physiological expression or function.
- 3. A gene is a unit of mutation.

Before the development of microbial and biochemical genetics, several attempts were made between 1900 and 1945 to understand the nature of gene. After the rediscovery of Mendel's work, certain cases came to light which indicated that in those cases heredity was not probably in accordance with Mendel's laws, for example, heredity of walnut, pea, rose and single combs in poultry. An attempt was made to explain it on the basis of the 'Presence and absence theory'. According to this theory, the dominant character of an allele depends on the gene present in it. It is absent in the recessive character. Taliness of tall pea plant is due to the gene for tallness. Absence of this gene results in the plant being dwarf. In other words, all plants are basically dwarfs but in the tall plant, there is a gene which makes the plant tall. Now we know that the explanation of such an example lies in the interaction of genes. When cases of multiple alleles, one genic or polygenic control and pseudoalleles came to light, they helped somewhat in our understanding of the nature of gene.

Serebrovsky worked on the presence and arrangement of bristles of head and thorax of Drosophila. He classified bristles into 12 types. He found that the gene scute is responsible for the length and arrangement and it forms an multiple allelic series. Each allele individually affects bristle hairs in different combinations. Serebrovsky described this action in term of the Theory of step-allelomorphism. This theory helps us to understand how differences exist party officer of at his reason party, which are the party sometime in multiple alleles.

Fisher (1928-32) proposed the theory of evolution of dominance. It is based on phylogeny and statistics. As compared to mutants, the wild type is dominant. In this case, modifiers are required for changing the phenotype of the heterozygote into the wild type because the wild type gets the advantage of selection. The concept underlying this theory is that the gene action here is similar to that occurring in the case of vestigial characters. This action is, however, slow. According to this theory, dominance as a specific trait will have to be sought in the already existing wild type. This theory helped to understand the nature of dominant genes in wild types. Similarly, when instances of polymorphism and position effect came to light, they added more to our knowledge of the nature of the gene to some extent.

In recent years, extensive information has accumulated in the field of microbial genetics and it is because of it, that our understanding of the nature of the gene and its structure has been very much increased. It has resulted in considerable modification of the classical concept of the gene.

We have already seen that genes exert their influence over the development of a character through enzymes. In other words, they are involved in the production of enzymes which are proteins. The structure of a specific protein is determined on the basis of information contained in the DNA molecule.

Chromosomes contain DNA which is the hereditary material. As per the classical concept, the gene is situated in the chromosome and is an ultimate unit of heredity. Therefore, it must contain DNA. Now the problem is whether the gene is equivalent to a DNA molecule or its part or to several DNA molecules.

Recent work, particularly those of Benzer, Bonner, Jacob and Monod, and Pontecarvo, has helped in solving the above mentioned problem and in understanding the fine structure of the gene and its action.

### Fine Structure of Gene

Benzer studied mutations and recombinations in the bacteriophage,  $T_4$ . In this phage, he found that there is a class of mutants called  $r_{\rm II}$  which can be identified with ease by the appearance of plaques. These are formed on a given bacterial culture. A plaque appears as a clear region on the surface of a culture in a petri dish where plaque particles have multiplied and lysed bacterial cells. This property enables one to count individual phage particles without even observing them. Besides, the shape and size of the plaques are hereditary features of the phage which can be easily scored. The standard form of  $T_4$  occasionally mutates to  $r_{\rm II}$  and these mutants are easily observed since they produce a distinct plaque on cultures. The key to the entire mapping techniques lies in the fact that these mutants do not produce plaques on K cultures. However, an  $r_{\rm II}$  mutant can grow normally on E. coli strain K if the cell is simultaneously infected with a particle of a standard type bacteriophage. It is because the standard DNA molecule is able to perform some function necessary in K, which the mutants are unable to do. This functional structure has been found to be a small portion of the DNA molecule which in genetic maps of the phage is termed the  $r_{\rm II}$  region.

Over 3000 individual  $r_{\Pi}$  mutants were isolated and studied by Benzer. On the basis of the classical concept, the  $r_{\Pi}$  region is to be considered as a gene and which should be a unitary

particle. However, Benzer's work on this phage gene convincingly shows that mutations occur within the gene itself. In other words, the gene is divisible into mutational sites. Benzer called each mutational site a muton.

The  $r_{II}$  mutants are divided into two groups which Benzer has designated as A and B groups (Fig. 31.1). Any combination of an  $r_{II}A$  and  $r_{II}B$  mutant produces the normal numbers of phage in  $K(\alpha)$ , phages of both the genotypes being formed. This means that the mutant types complement one another to allow growth. On the other hand, any combination of  $r_{II}A$  with  $r_{II}A$  or  $r_{II}B$  gives rise to a few, if any, or none normal phage particles. By analogy with the phenotypic tests of diploid organisms, two  $r_{II}$  regions are designated by Benzer as the gene A and gene B on the basis of the functional criterion. All A mutants are functional alleles and so are the B mutants. However, A and B functions are interdependent of one another. Benzer, therefore, termed them as cistrons. A cistron consists of several mutons.

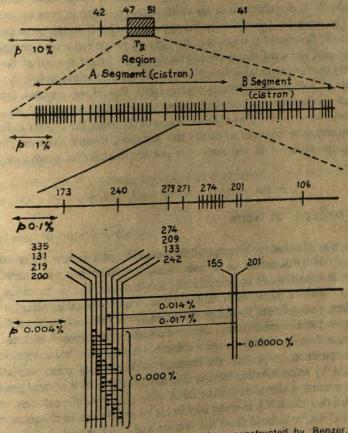


Fig. 31.1 Genetic map of  $r_{II}$  region in  $T_4$  phage constructed by Benzer. p=recombination frequency. m, tu and r-minute plaque, turbid plaque and rapid lysis mutants, respectively. (After Benzer, Brookhaven Symp. in Biol. No. 8, Mutation, pp. 4 (1957).

All  $r_{II}$  mutants are closedly linked. When Benzer crossed  $r_{II}$  mutants, he found that  $r^+$  recombinants were produced not only in  $r_{II}A \times r_{II}B$  crosses but also in  $A \times A$  and  $B \times B$  crosses. Crossing is dependent on using two different  $r_{II}$  mutants to infect bacteria in which the  $r_{II}$  mutants can grow, e.g. E. coli strain B. These results point out that there also occur intragenic recombinations. So Benzer has suggested a unit of recon on the basis of recombination. In order to have recombination, two mutons will be necessary.

Benzer has mapped different r mutants and the resulting map is linear (Fig. 31.1). All the A mutations are located in one part of the map and all the B mutations in an adjacent section. There is no overlapping of the positions of A and B mutations. The A group and B group are functional units. So the functional unit of the genome is larger than the recombination unit. Many mutations which act as functional alleles are found to be separable by recombination. Hence, a group of functioning alleles seems to define a discrete segment of the genome which is divisible by recombination, i.e. recons, rather than defining one individual particle. It is divisible into larger units, cistrons, which in turn are made of mutons and recons. The work on Asperqillus or Neurospora also leads to the same conclusion.

A cistron is the functional equivalent of the gene. It is applied when complementation is the only functional test used. Some geneticists, however, prefer to restrict the term gene for describing only the fundamental indivisible units of recombination, recons. So another classical term, allele, in unis context becomes controversial. Should we use this term to refer to elements which are functional alternatives (alternatives at the same rate)? As per the original definition, alleles were regarded as different states of the gene, the former definition appears to be quite appropriate. In order to clarify this situation, Romen has used the terms homoalleles for genes differing at the same site and heteroalleles for genes having mutation at different sites.

## Modern Concept of Gene

Let us now sum up the modern concept of the gene. The gene is now regarded as consisting of a segment of DNA that serves as a code for a particular protein. Therefore, the gene must be linear in structure and consist of many sub-elements, i.e. nucleotides. A nucleotide is the functional unit of mutation or muton. If recombination takes place within a DNA molecule, adjacent nucleotides should be separable by recombination. Therefore, we have to equate the element of recombination, recon and mutation, and muton with the nucleotide of a DNA molecule. Because each gene is considered as acting as a 'transcription unit' in the synthesis of its product, i.e. protein, the fundamental allelism of genes consisting of mutations separable by recombination implies that the formation of a normal gene product can be prevented by causing alterations in the DNA code at many different sites within a gene.

Bonner states that the DNA in some way, must be condensed in chromosomes, since the total length of DNA, calculated from the Watson and Crick model, far exceeds the actual chromosome length. He has suggested that the DNA is arranged in cistrons in a tree-like style with the DNA as branches along a protein trunk. Reciprocal recombination then could occur along the trunk and non-reciprocal recombination along the limbs, but again this is only a speculation. The hard facts are still to be discovered.

# Regulation of the Gene Action

Our knowledge about the regulation of gene action in higher organisms is very little. It is, however, now clear that genes are regulated and they function in specific tissues. The orderly process of the development of the embryo and its differentiation supports this contention.

# Feedback Inhibition (End Product Inhibition)

It is now understood that in the case of a number of biosynthetic pathways, the accumulation of the final product of the reaction is capable of inhibiting the first step in the reaction. This is indicated in the diagram below.

$$A \rightarrow B \rightarrow C \rightarrow \rightarrow P$$

The end product P inhibits the enzyme which converts A into B. This is not simply mass action, as is indicated by the failure of intermediates to accumulate. In some cases, it is found that the end product acts by altering the enzyme, possibly in its three-dimensional structure, with the result that it becomes temporarily non-functional. However, the organism has to carry all the enzyme proteins irrespective of whether they are being used or not. It might be more efficient in controlling the actual synthesis of the enzyme and then the cell would not be required to carry around any excess of enzymes which are not required. In fact, such a regulatory system does exist.

## Suppressor Genes

These genes inhibit the phenotypic expression of other genes. They may not be linked to the gene whose action they suppress. Their action is possibly indirect.

# Structural and Regulatory Genes

We already know about genes whose function is to determine the structure of a protein. Such genes are called structural genes. However, there are also genes which regulate the action of other genes which specify the structure of particular genes. These are known as regulatory genes. Mutation of a regulatory gene can result in a marked change in the total amount of enzyme produced by the cell. A regulatory gene has been recorded to control the formation of tryptophan synthetase in E. coli. There is also another gene which regulates the production of beta-galactosidase in the fruitfly. The regulatory gene in these cases has been found to act in such a manner that it turns a structural gene 'on' or 'off'. One regulatory gene can co-ordinately repress the production of several functionally connected enzymes.

The French microbiologist Francis Jacob and his coworkers (Monad, etc.) carried out work in connection with the regulation of gene action. Based on their work, they have suggested what is called the operon concept. In order to explain it, let us assume an active structural enzyme as enz+, its alleles enz-, the operator as op+ and its allele op-. It is necessary to have operator genes in close linkage for function. They may regulate directly at the gene level with no intervening cytoplasm. In this case, there are two possible heterozygous diploid combinations as indicated below.

Only the configuration which is of the cis type allows enzyme formation. Hence, the regulation by the operator gene needs linkage as well as cis configuration. These results as well as other data obtained by Jacob and his associates lead to the conclusion that the operator gene can directly regulate the formation of the messenger RNA of the structural gene. Operator genes like regulatory genes seem to act by a mechanism which does not need the operator to form a particular enzyme. One operator gene may regulate the formation of functionally connected enzymes subject to the condition that the structural genes are themselves linked. For instance, the genes concerned with regulation of the biosynthesis of histidine are linked and all are controlled by one operator. The genes which control the production of beta-galactosidase and the permease required for the lactose uptake are linked in E. coli and both of them are controlled by the same operator gene. On the basis of these findings, Jacob and Monad proposed a new genetic unit called l' operon. This unit is a package deal consisting of an operator gene and the genes it regulates (Fig. 31.2).

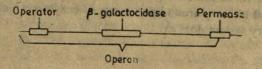


Fig. 31.2 Operon: No Memory and Marian

Many workers are now engaged in defining the molecular basis of the operator gene action. Their data and new concepts indicate that the genetic material may be existing in two distinct types. One determines the structure of formed enzymes while the other regulates the rate of process of enzyme formation. It is still not known whether the genetic material needed for these different kinds of gene action is the same or different.

#### SUMMARY

- 1. Before the development of microbial and biochemical genetics, several attempts were made, between 1900 and 1945, to understand the nature of the gene. It is possible to study the gene with standard genetic techniques, by observing recombination, phenotype and mutation. The classical concept of the gene envisages the latter as a unitary particle not sub-divisible by chromosomal breakage or crossing over.
- 2. Genes contain DNA which is the hereditary material. Now, the problem is whether the gene is equivalent to a DNA molecule or its part or several DNA molecules.
- 3. Benzer studied mutations and recombinations in the bacteriophage  $T_4$ , using very refined techniques. On the basis of this work, he was able to describe the fine structure

of genes. He found that there is a class of mutants called r<sub>II</sub> in the phage. He convincingly showed that mutations occur within the gene itself. He called each mutational site a muton. The r<sub>II</sub> mutants are divided into A and B groups. All A mutants are functional alleles and so are the B mutants. However, A and B functions are independent of one another. Benzer termed them cistrons. A cistron consists of several mutons. All r<sub>II</sub> mutants are closely linked. Benzer crossed these mutants. He found that intragenic recombinations also occur. He has suggested a unit of recon on the basis of recombination.

- 4. According to the modern concept of the gene, it is a segment of DNA that serves as a code for a particular protein. The gene must, therefore, be linear in structure and consist of many sub-units, nucleotides. A nucleotide is the functional unit of mutation or muton. Because each gene is considered as acting as a 'transcription unit' in the synthesis of protein, the fundamental allelism of genes consisting of mutations separable by recombination indicates that the formation of a normal gene product can be prevented by causing alterations in the DNA code at many different sites within a gene.
- 5. Genes whose function is to determine the structure of a protein are called structural genes. The genes which regulate the action of other genes which specify the structure of particular genes are known as regulatory genes. Jacob and Monad have put forward the operon concept to explain gene action. The unit "l' operon" is a package deal consisting of an operator gene and the genes it regulates. The regulation by the operator gene needs linkage as well as cis configuration. The operator gene can directly regulate the formation of the messenger RNA of the structural gene.

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# 32. Genetic Engineering

The science of genetics has made such tremendous progress that in the near future it would be possible to modify the genetic constitution of an individual through genetic manipulation, i.e. genetic engineering. Of course, there are a lot of technical difficulties which are to be overcome. However, the advent of genetic engineering has opened up exciting possibilities which include (i) the application of a relatively quick and accurate method of incorporating specific and desirable genes into an organism and (ii) the transfer of genes between sexually incompatible species. For the application of these techniques, transfer of embryo is likely to prove a convenient vehicle.

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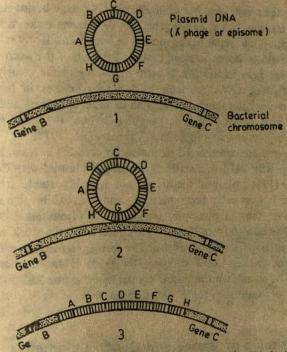
Genetic engineering can be defined as the process whereby a foreign sequence of DNA is inserted into the genetic make-up of a cell by in vitro techniques, and is subsequently expressed by that cell (Ingle, 1982).

### Recombinant DNA

We have already learnt about episomes which possess the general property of existing independently of chromosomal DNA or becoming integrated with the chromosomal DNA. In the independent stage they are usually small circular molecules. They are also called plasmid DNA. The lambda ( $\lambda$ ) phage DNA is an excellent example of plasmid DNA. It remains either free of the chromosomal DNA of E. coli or becomes integrated with it. Campbell has proposed a model to explain this integration of plasmid DNA. There is first synapsis or pairing of plasmid DNA with chromosomal DNA followed by recombination whereby there is insertion of plasmid DNA into the chromosome (Fig. 32.1). So the plasmid DNA is called the recombinant DNA. This is now being used in genetic engineering.

The most popular technique for the preparation of a recombinant DNA involves fragmentation of the parental DNAs by specific restriction endonucleases. This is followed by joining the fragments by DNA-ligase. In this way, a set of different molecular forms, circular and linear oligomers composed of a different number of fragments (Debabov, 1981) is produced.

Plasmids in the majority of cases are not the essential constituents of a cell. However, they are responsible for a set of clearly defined physiological characters such as fertility, resistance to antibiotics, etc. Their ability to replicate autonomously and some phenotypical properties which they possess have made them an important tool of genetic manipulation.



Plasmid DNA or episome. 1. Circular DNA is A Fig. 32.1 phage (plasmid). Below it, a part of the bacterial chromosome is shown. Plasmid DNA is to pair with this part. 2. Recombination between plasmid DNA and the bacterial chromosome, 3. Integration of plasmid DNA into the bacterial chromosome due to recombination. (Redrawn from Woodward and Woodward, Concepts of Molecular Genetics, 1978).

## Vectors

Vectors provide the recombinant DNA molecules an opportunity of their being introduced into the host cell and thus acquiring the ability to replicate and maintain themselves with stability in generations, to come. Vectors must possess the following:

- 1. Properties of replication, and sometimes, of transcription.
- 2. Substrate sites for restrictive endonucleases so that they are able to integrate foreign DNA segments.
- 3. One or more genetic markers so that necessary prerequisites for the cloning of recombinant DNA molecules are produced.

Plasmids, temperate and some other phages generally make use of the replication apparatus of their host cell. Only a few proteins essential for the replication of a plasmid or a phage are coded by their geno nes. For example, \$\ppreceq X\$ 174 phage begins its replication so that the particular endonuclease coded by the gene A of its genome makes a nick at or near the origin of the replication and remains for some time bound covalently to the 5' end of the nicked DNA. It should be borne in mind that the function of the vector is not just replication. It also involves gene expression. Recent researches have clearly indicated that the expression of recombinant DNA molecule of lower eukaryotes in heterologous systems does occur; for example, heterologous expression of genes of leucine and histidine synthesis in E. coli (Strupl and Davis, 1977; Ratakin and Carbon, 1977) and genes of quinic acid metabolism in Neurospora crassa and E coli (Vapnek et al., 1977).

Most plasmid vectors are constructed by molecular crossing in vitro. Some of them can be made in vivo, e.g. RSF 2124 was made by crossing R plasmid with ColEl (Bayev, 1981). According to Bayev (1981), plasmid vectors are not probably stable structures. They can combine and mutate in host cells. Some parts of them even exhibit segregation.

Bayev (1981) states that in principle, integration of recombinant DNA into the cell is quite possible. He has quoted from Fink's (1976) papers and the work in his laboratory by Tikhomicova and Strijov in support of this. The former transferred a leu-strain of yeast with the recombinant plasmid ColEl carrying the yeast DNA frequent leu+. The latter workers integrated a recombinant molecule consisting of vector RSF 2124 (Apr Colimm and Eco Rl) frequent C of phage into the E. coli chromosome.

# **Artificial Synthesis of Genes and Control Elements**

It has now become possible to design and synthesise genes and control elements that can be integrated to plasmid elements in order to bring about replication in E. coli and other bacteria because of the advancement in techniques in respect of chemical synthesis of DNA and recombinant DNA. Khorana and his colleagues synthesised the gene, yeast alanine transfer RNA, in 1970. This was the first in vitro synthesised gene. It contained only 77 nucleotides. However, for synthesising a human gene, millions of nucleotide pairs will be required and even a single error in respect of a pair could bring about disastrous results. In 1975, Khorana, Fritz and Ramamurty Balgaje replicated a gene of E. coli in their laboratory. It contained about 200 nucleotides. This progress in antificial synthesis of gene containing many nucleotides promises that in some years it would be possible to synthesise genes in vitro consisting of innumerable nucleotide pairs.

The artificially replicated gene in E. coli was introduced in 1976 in a living bacterial cell. It was seen that it was functioning quite normally. In 1930, Cline, Salcer, Marcola and Stang synthesised a gene resistant to drug-resisting cancer. They successfully introduced it into the bone marrow cells of mouse. They found that the artificial gene was carrying on its assigned function successfully. Some diseases are caused because of gene mutations. These genes are, therefore, unable to carry on their normal functions for which certain enzymes are necessary and which are not produced because of mutation. If a gene responsible for the formation of a specific enzyme is synthesised, then it would be possible to control the disease caused by mutation and even eradicate it.

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## **Nuclear Matrix**

Recent researches have indicated that for the synthesis of messenger RNA only one-tenth of the total DNA molecular chain is active and the rest controls the activities in the nucleus: Scientists have now turned their attention to this part of DNA. In 1980, Pardoll and Hogelstein discovered a skeleton-like structure which they called the nuclear matrix. According to them, this structure represents an 'apparatus of gene nature'. The objective of such researches is to find out ultimately when the gene is active and when its activistops. When this information become available, it would be possible for scientists to start the gene action or to stop it.

# Some Achievements

We have seen in the chapter on mutation that mutagenic substances can cause substitution of nitrogen bases in the DNA molecule or to produce deletions. Recently, it has been possible either to delete an entire gene or introduce it in a cell. In 1980, Ruddle, Gorden and Scangon injected a viral gene into the just-fertilised egg of a mouse. This injected gene was permanently integrated into the embryonal cells of the mouse thus altering the hereditary constitution of the mouse.

The discovery of recombinant DNA has given a fillip to the progress of genetic engineering to a large extent. Attempts are being made with its help to improve agriculturally useful soils from the point of nitrogen fixation. Experiments are being made in respect of genes involved in nitrogen fixation (nif operon) present in specific bacteria and their transgenes involved in nitrogen fixation (nif operon) present in specific bacteria and their transference to the bacteria devoid of them. If these experiments are successful, the problem of nitrogen fertilisers would be solved to a large extent.

Proper splicing of the spiral of the DNA molecule and joining the parts whenever necessary has now become possible. This technique can be employed in making different gene combinations. Of course, such combinations can be beneficial or even harmful. Some express fear that unregulated use of this technique might result in creating monsters. Hence, express fear that unregulated use of this technique might result in creating monsters. Hence, express fear that unregulated use of this technique of regulations in the case of the opinion in the USA is gathering strength to have certain regulations in the case of the technique of recombinant DNA is clon-

The most important step in the progress of the technique of recombinant DNA is cloning. Injecting a new gene in a bacterium and to make it stabilised amounts to cloning, i.e. ing. Injecting a new gene in a bacterium and to make it stabilised amounts to cloning, i.e. is self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hybrid plasmid' in a foster mother (Fig. 32.2). After introducing self-reproduction of the 'hy

In the bacteriophage o3. T, the gene necessary for thymidelate synthetase (thy 1.) is present. It can be integrated in the chromosome of Bacillus subtilis. After integration, the strain requiring thymine is transformed into a strain independent of thymine requirement. In strain requiring thymine is transformed into a strain independent of thymine requirement. In this way, the use of entire bacteriophages or thy P<sub>3</sub> can be made in a kind of genetic surgery for the transference of heterologous DNA.

#### 348 Cytology and Genetics

Techniques are now available to introduce new characters by transferring heterologous genes into bacteria. It is now possible to increase the biosynthetic potential at a desired level in a cell by increasing the gene dose. It will be interesting to study the functions of the constitutive genes in the heterologous bacteria with the help of genetic engineering.

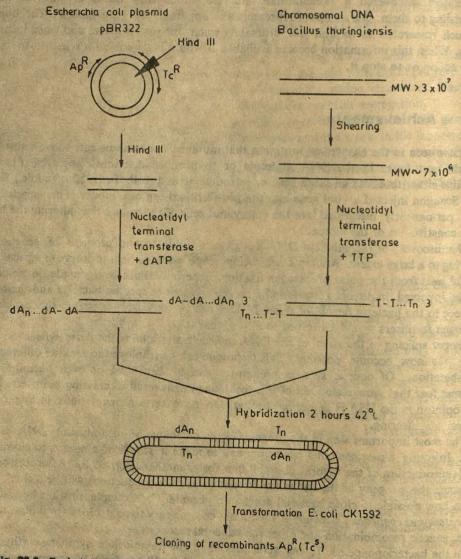


Fig. 32.2 Technique used in the insertion of pieces of DNA obtained by shearing with the help of nucleotidyl terminal transferase in the plasmid of Boyer p BR 322 cut in the gene for tetracycline resistance by the endonuclease Hind III. (After R. Dedonder et al. Molecular bases of genetic processes, Proc. XIV Internat. Genet. Cong. Vol. III, Book 2, 1981, pp. 194, Table 4).

Bean belongs to the family Leguminosae whereas sunflower to the Compositae. These two families are not at all closely related. Hence, it is not possible to cross them successfully by the conventional methods. However, such a cross has been made successfully by employing the gene splicing technique. It appears from the statement published by the German Foundation for International Development that during June 1981, a team of scientists transplanted a gene from a bean plant into a cell of a sunflower plant to get a cross called 'sunbean'. An intermediary bacterium Agrobactor tumefaciens was used for transferring the gene from the bean plant to the nucleus of a sunflower cell. It has been claimed that the gene has stabilised in its new environment. The bacterium causes tumours in plants. So scientists are trying to inhibit the tumour formation. If this wide cross becomes successful and the hybrid sunbean produces offspring, the science of plant breeding will be revolutionised. It will lead to successful crossing of any two desirable plants which may not be at all closely related.

In 1981, Dr. A.M. Chakrabarty, an Indian Scientist at Chicago, claimed to have produced an entirely new strain of microbe using genetic engineering techniques. This new strain does not occur in nature. He collected microbes which had already begun developing an ability to degrade complex chemical molecules. He fed them with a special plasmid which helped the microbes further to break up certain molecules and so develop into what he called Pseudomonos cepacia or PC. According to Chakrabarty the soil can be decontamina-

ted by applying these new microbes once a week for six weeks.

In 1981, Reddy, Smith and Aaronson deciphered the genome of the Moloney mouse sarcoma virus. This virus is known to cancer researchers. It is a type C RNA retrovirus. The same kind of virus causes many known animal tumours and can be transmitted from parent to offspring in inherited genes. This virus contains a chain of 5828 base pairs. Their exact sequence is now known. The chain appears to have 'hot spots' where it can combine with genes in cells to become the transforming genes that may lead to a cancer.

One of the exciting features of genetic engineering is in relation to microbial breeding. It has now become possible to synthesise small protein hormones by bacteria and some positive results have already been obtained in the case of somatostatin as a part of β-galacto-

cidase and insulin as a part of penicillinase (Sermonti, 1981).

Latest information indicates that laser, the device developed by physicists for producing intense and sharp beams of light, has become a versatile tool in the hands of biologists. Scientists working at the Institute of Physics and Chemistry, Tokyo, have developed a technique of using the laser beam to drill a hole in the membrane of a living cell and introduce a DNA molecule into it. In this technique, two DNA molecules are brought together in a test-tube so that they combine to produce a new DNA molecule. It is claimed that this technique will enable scientists to modify genetic characteristics of a species ten times more efficiently than by the present methods. No damage is caused to the cell in this technique.

Fermentation, enzymes and recombinant DNA offer a possibility of technological revolution in fields such as health, energy, food, mining, agriculture, industrial chemicals and environmental sciences. It is now possible to synthesise many necessary substances, e.g. amino acids, vitamins, enzymes, antibiotics, synthetic vaccines, diagnostic reagents, fuels, 

valuable human protein interferon, etc.

### **Protoplast Fusion**

It is now possible to remove the cell wall of bacteria, fungi, yeast and other plant cells under proper conditions without influencing the viability and integrity of the cell as a whole. When the cell wall is removed, a unit surrounded by the plasma membrane is obtained. It is spherical in shape in hypertonic media and is called the *protoplast*. When the appropriate conditions are provided, the protoplasts can be induced to resynthesise the cell wall and thus revert to its original form.

Kao and Michaylul in 1974 showed that polyethylene glycol (PEG) is a very active agent for the fusion of plant protoplasts. Since then PEG has been used successfully in inducing fusion of protoplasts in many microbial systems.

In order to obtain a complemented or recombinant microorganism through protoplast fusion, at least three processes in the fused parental protoplasts (Alfoldi, 1981) take place. These are (i) cytoplasmic interaction, (ii) DNA-DNA interaction, and (iii) reversion to microbial form.

When the membranes of the parental protoplasts are disrupted at the union contents of protoplasts, they freely mix with each other. However, since the fine structure and integrity of each cytoplasm is to be maintained, certain procedures involving environment at cytoplasmic interactions are adopted. In fused protoplasts, there may be interaction between whole genomes via complementation or recombination or both.

Protoplast fusion has opened new vistas in genetic engineering. It offers new possibilities in the construction of microorganisms for industrial purposes.

### **Tissue Culture**

Advanced techniques in tissue culture are being exploited as a tool in genetic engineering. Animal tissue cells in culture are now transformed by viruses like simian virus-40 and papilloma viruses. When cultured mouse or human cells are treated with 3U-40, viral DNA gets incorporated in their chromosomes. When foreign genes are injected into the male nucleus, they are frequently incorporated into the mouse chromosomes. If such a nucleus is involved in fertilising the egg of a mouse, the new genes acquired are expressed in the mouse throughout its life and moreover, they are inheritable.

# AND BUILDING TO BERRY AS STORES OF SUMMARY

1. Episomes possess the general property of existing independently of chromosomal DNA or becoming integrated with chromosomal DNA. They are also called plasmid DNA. The lambda phage DNA is a plasmid DNA. In its case, there is pairing of plasmid DNA with chromosomal DNA of Escherichia coli and then follows recombination whereby there is insertion of plasmid DNA into the chromosome. The plasmid DNA, known as the recombinant DNA, is used in genetic engineering or surgery.

- 2. Vectors provide the recombinant DNA molecule an opportunity for being introduced into the host cell and thus acquiring the property of replication and maintaining themselves with stability in generations. Most plasmid vectors are constructed by molecular crossing in vitro.
- 3. It has now become possible to design and synthesize genes and control elements which can be integrated to plasmid elements in order to bring about replication in Escherichia coli and other bacteria due to the advance in techniques in respect of chemical synthesis of DNA and recombinant DNA. The artificially replicated gene in E. coli was introduced in 1976 in a living bacterial cell and it functioned normally.
- 4. Some diseases are caused by gene mutation. If a gene responsible for the formation of a specific enzyme is synthesised, then it would be possible to control the disease in such cases and even eradicate it.
- 5. Discovery of recombinant DNA has given a fillip to progress in genetic engineering. Attempts are being made to transfer genes involved in nitrogen fixation to nonleguminous crop plants. Proper splicing of the spiral of the DNA molecule and joining the parts whenever necessary has now become possible. This technique can be employed in making different gene combinations. The most important step in the progress of the technique of recombinant DNA is cloning. Injecting a new gene into a bacterium and to make it stable amounts to cloning. Techniques are now available for introducing new characters by transferring heterologous genes into bacteria. Fusion of protoplasts and tissue culture are also employed in genetic engineering, NEW TOTOTOPINE SEE TO SOME ASSESSMENT STREET the spirit of the second transfer of the second second to the second sec

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# 33. Genetics and Breeding

The basis of the breeder's work is heredity. Hence, knowledge of genetics has always helped both the animal and plant breeders.

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## **Historical Background**

The history of breeding like the history of genetics is related to the history of human civilisation. It did not begin, as a science. When man gave up the life of wandering and settled down at one place, he felt the need of improving the quality of his domestic animals. He started cultivation of plants which could give him food and cloth. For cultivation, he required good plants and animals. So he started selecting the best among the crop plants and animals, and bred them. The history of plant and animal breeding is, therefore, vary old. The Arabian horse, Merino sheep, some varieties of rice, wheat and maize represent the ancient varieties. The historical record of China indicates that many varieties of rice were cultivated even before 5000 B.C. The excavations at Mohenjodaro point out cultivation of cotton in India before 5000 B.C. Wheat grains were found in the Egyptian mummies indicating that wheat was cultivated by the ancient Egyptians.

Though ancient man practised selection of plants and animals, their breeding had no conscious and scientific basis. Though sex in animals was known at the time, sex in plants was, however, unknown. In 1676, Nemiah Grew stated that stamens represented male sex. In 1694 Camararius proved with the aid of the microscope that sex is present in plants like mulberry, maize and caster-oil plant. In 1760, Kolreuter was the first to practise artificial hybridisation in plants. Knight (1759-1838), Goss (1822), Naudin (1863), etc. used hybridisation technique for improvement of plants. However, a scientific basis for animal and plant breeding could be available only after the rediscovery of Mendel's work in 1900, since his work laid the foundation of the science of heredity which forms the basis of the breeder's work.

We know heredity is, in fact, a question of individuals. Similarity in phenotype is not a sure criterion to reproductive qualities. Two individuals though bred similarly and indistinguishable in the external form may behave differently when bred from. For example, in sweet pea, the  $F_2$  generation consists of seven distinct types, of which there are three sets of purples, three kinds of reds and white. Let us assume that our object is to get a true breeding strain of pale purple picotee. We know on the basis of the proportions in which they are obtained that dilute colour is because of the absence of the factor responsible for intensification of colour. Hence, picotees are unable to throw the two deeper shades of red or purple. However, it

may be heterozygous for purple factor, in which case it will give rise to dilute red (tinged white) or it may be heterozygous for either or both of the two colour factors, when it forms whites. From the picotees derived in this way, some will throw picotees, tinged whites and whites, others will perhaps form only picotees and whites, whereas others again, and these the least numerous, will give only picotees. The new variety is already fixed in a certain definite proportion of the plants. In this case, for example, one out of every 27 is fixed as the new variety. What is to be done now is to pick out these plants. Since all the picotees have some appearance irrespective of their breeding ability, the only way to do this is to save the seed from a number of such plants individually and to have the further generation. In this way, the variety is then established. It may be released at once into the market with full confidence that it will breed true. The most important point is to save and sow the seed of separate individuals separately, because heredity is a question of individuals. A breeder who understands this will save much labour and be able to establish his varieties in the shortest possible time.

The keynote of the breeder's cooperation is the improvement of a specific crop, His object is the production of a strain which shall combine the largest number of desirable properties with the least number of undesirable ones. He must concentrate on a good quality in one strain, others in another strain and so on and take care to avoid all poor characters the different strains exhibit. This he can learn from the Mendelian conception of characters which are carried by the genes which are transmitted on a certain definite pattern. When these factors are ascertained, their distribution can be brought under control. They can then be associated together or dissociated as per the desire of the breeder. What is involved chiefly is the labour required to determine the factors on which different traits depend. The streets gold and, Endowed Bent Departure (second age ) took off the

# Animal Breeding

Even before 1900 the art of animal breeding was well advanced. Behind this progress, there lies the history of several centuries of trial and error made by men. As regards improvement of domestic animals, the breeder succeeded in certain cases to introduce characters desired by him. However, very little was known about the scientific reasons of the occurrence of events in such cases.

In the nineteenth century, pure breeding was established as the standard method to produce seed stock. This was accompanied by the introduction of herd books and breed associations. These served as mechanisms to maintain purity of breed and to take other cooperative actions necessary in the interest of breeders. arrian grafts of metarina multi-pic to the last specimes and son vine at a few

Pedigree Method Cross-breeding and grading were practised on a large scale for producing commercial stock. The principal method of improvement was selection within pure breeds. The basis of selection was chiefly the merit of an individual. Attempts were made to use pedigrees in estimating the worth of an animal. They led the users to try to have the pedigree mean more than it possibly could. The first principle of animal breeding was: Like begets like. The second principle was: Like does not always produce like. Some authors of this period and even up

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to 1920 contrasted the 'law of heredity' with the 'law of variation' and concluded that they were opposite or mutually exclusive forces.

#### Impact of Mendelism

The impact of Mendelism and immediately subsequent researches was chiefly in three directions. First, it became clear that identical pedigrees do not necessarily mean identical heredity. Secondly, it was realised that both the genetically-caused and environmentally-caused variations were present and these were frequently indistinguishable in an individual but, nevertheless, showed quite different results in the case of their descendants. Thirdly, it became evident that there was no need to assume that mutations were so abundant or that their nature or causes were so important in the case of results of practical animal breeding as had previously appeared obvious and essential.

#### **Progeny Testing**

In the beginning, progeny testing was rather unsystematic but as experience grew and the consequence of Mendelism, biochemistry and environment became clear, it became precise. When it was realised that the samples must be representative if they were to be used without any bias, non-selective systems of testing were devised. The majority of breed associations adopted them and by 1930 became very popular. The need of the progeny sample or sibs being unselected would not have been understood had not geneticists already been engaged in solving the problem of testing an individual for homozygosis where dominance is complete.

Some breeders were curious about the probable results of various mating systems. In spite of the fact that Mendel himself had worked out the effects of selfing on single pair of genes, the early Mendelian workers were slow to apply this to many pairs of genes and to the milder systems of inbreeding in animals. The attempts of Pearl made in the beginning of 1912 and more complete analysis made by Jennings a few years later helped in the clarification of the underlying principles to some extent, but genetic theory hardly overtook the practice of animal breeding as regards this aspect until the publication of the work of East and Jones (1919) on inbreeding and outbreeding. After Wright's monumental work on mating systems (1921), it became possible to make a more rational use of mating systems, particularly line breeding and cross-breeding. Wright (1922) developed a measure of the possible Mendelian effects of such matings and it enabled a breeder to compare his results with theoretical ones.

#### Selection Index

The most effective device of selection is the computation of an 'index' which combines in a single figure as many possible complex bases for deciding whether to call an animal or to use it in a breeding programme. This object estimate of the breeding worth of an individual consists of information obtained from all possible sources of evidence about it. Each source is assessed in accordance with its relative importance to the breeder's objectives.

finitely utilizes the appearation the being sport another or

There is another as act which also requires attention of a breeder while evaluating the characteristics to be selected. He has to find out the degree to which each of them is heritable. In other words, the extent to which variation in successive generations can be predicted in terms of genetic control and therefore to be subjected to selection in a straightforward

way, is to be determined, i.e. heritability. In practice, this straightforward genetic control in most cases of selection refers to variation which can be regarded as the result of additive gene effects.

The computation of a selection index forms a major part of a breeding programme. However, there are yet only a few cases in which sufficient information is available to compute approximately a complete index. While using mutagenesis in a breeding programme, it should not be forgotten that it is not, at present, possible to control mutation. A breeder may, however, be able to utilise desirable mutations or weed out undesirable ones more quickly than in nature by selection and control of the mating system. He can control migration. The purchase of new male or breeding females introduces new alleles and new gene combinations into the herd.

## Coefficient of Inbreeding

An animal breeder expects to make progress with the help of two primary tools: (i) control of selection in his herd of flock, and (ii) control of its mating pattern. For achieving this, he has to pay attention to the genetic relationship which is a variable quantity and for which he requires some kind of quantitative measure. Wright's coefficient of inbreeding provides this measure very well. It measures the degree to which animals may possess genes in common due to their descent from the ancestral individuals.

Individual breeders should not take chances in fixing rapidly truly inbred lines. They have to experiment with milder inbreeding, if the advantage of desirable genetic effects of this genetic system is to be availed of and no risk is to be taken of its adverse effects.

This is a kind of inbreeding involving intensification of the contribution of particular desirable individuals to the herd or flock while maintaining the relationship among contemporary individuals at a minimum. This form of inbreeding is of a long standing value in the improvement of an animal. In the case of domestic animals, the characters like productivity and reproductive potential which involve continuous variation, a variety of different patterns is necessary for execution of practical programmes aimed at improvement of domestic animals.

## **Plant Breeding**

There is an intimate relationship between plant breeding and genetics. It was soon realised after the discovery of Mendel's laws that they would have far reaching consequences in relation to practical plant breeding work.

The principle of genetic recombination clearly indicated to the breeders the possibility of combining various characters from different parent strains. In fact, genetic recombination forms one of the corner-stones of the modern science of plant breeding. It becomes obvious that Mendel's hereditary units, genes, were also fundamental units in the case of plant breeding. To the stable most appearant of the stable of

# Pure Line Experiments of Johannsen

The experiments performed by Johannsen, a Danish scientist, on selection proved to be instructive to plant breeders. He started his experiments in early 1900 to test some of the ideas about selection prevalent at the time.

Johannsen chose the Princess variety of garden bean and investigated the possibility of selecting for seed weight. He began with a mixture of seed obtained from several different plants. Initially he observed that progenies derived from heavier seeds generally had greater average seed weight than progenies derived from lighter seeds. So it was evident that selection was effective in this case.

Johannsen investigated the problem further with a refined experimental approach. He selected 19 seeds, each derived from a different mother plant, and grew them into 19 progeny plants which produced their own seed lots. These were kept separated within each seed lot. There was variation in weights from one individual to another. This variation fell into arrays nearly approximating normal distribution. Nineteen separate lines were propagated by Johannsen by selecting from each seed lot the heaviest and lightest seeds. He followed this procedure for several generations and noted that there was a remarkable constancy in weight average within each line, generation after generation, irrespective of the line produced by its heaviest or lightest seed. When selection was done for six generations in line 19, it was observed that the smallest parent seed produced a progeny having an average seed weight of 37 centigrams and the largest parent seed also produced a progeny with the same average weight.

Johannsen was dealing with beans which are self-fertilising plants. Inbreeding causes homozygosis. So in Johansen's second series of experiments, the parent beans with which trials started were homozygous. The progeny of any individual bean could not therefore exhibit any genetic segregation. They formed what Johannsen called *pure line*. He showed convincingly that selection is only effective as long as the selected material is made of different biotypes. Selection within a pure line is ineffective even if it is carried on for many generations. Later on, other workers confirmed his conclusions.

The constancy of genes also explained the constancy of clones wherein the same individual and the same genotype are reduplicated in the process of vegetative propagation.

The work of Johannsen led to the realisation that the isolation of new varieties by producing progenies from single individuals, could be carried out successfully only in the case of populations of self-fertilising species. However, in these cases also the method had its

## Theory of Multiple Jenes

Nilsson-Ehle (1906) pointed out the great difficulty involved in obtaining good products by line selection in respect of spontaneous populations. So he recommended the method of artificial crosses as the best for combining various desirable characters. In 1908-11, he showed convincingly that quantitative characters are usually inherited in a Mendelian fashion but as a rule, in this type of inheritance, many so-called polymeric genes or multiple factors are involved. This theory of multiple genes is of prime importance to plant breeders.

The frequent occurrence of both positive and negative transgression offers strong support in favour of the multiple gene theory. Quantitative characters in such instances are

conditioned by several or many constant genes which are recombined and the parents possess different sets of such genes.

The frequent occurrence of transgressions has been found to be very useful to plant breeding, for instance, yield in the case of 'Eagle oats'. Akerman produced this variety by crossing Lockows Gelbhafer with Victory oats. If the yield value of parents is 90 and 100 respectively, that of Eagle oats is 106, thus transgressing the parent value by 7%. these occurring productions. The raic of the latter had of monations is, in forth accelerat-

## Hybrid Corn and Heterosis

An excellent example of practical application of knowledge obtained from experiments carried on inbreeding and outbreeding is provided by the work of Shull and East on maize. Shull observed that if two parents were crossed, the hybrid progeny exhibited vigour. He (1914) used the term heterosis for hybrid vigour. This term is a kind of abbreviation of the word heterozygosis. It has been found that hybrids derived by crossing two inbred lines usually display vigour. They are remarkably more vigorous as compared to their parents. The discovery of hybrid vigour in maize or corn led to the extensive cultivation of hybrid corn in the USA. It revolutionised the economy of this country resulting in its great prosperity. In India also hybrid jowar (Figs. 33.1 and 33.2), hybrid corn (Figs. 33.3 and 33.4), hybrid wheat and some other food crops are now extensively cultivated.

The main reason for the success of hybrid corn lies in the fact that the members of an  $F_1$ hybrid group always exhibit a uniformity as regards high level of performance which is not seen in the case of open-pollinated varieties. After the  $F_1$  generation, characters like height and yield, however, are not maintained at such a high and uniform level because of segregation and  $F_2$  being more variable. This is the reason why farmers cannot use the seeds of hybrid corn or hybrid jowar. He has to purchase the seed produced from the cross or inbred lines or prepare his own seed separately. plant to vigor the great of terrestable and the property age of the property o

Overdominance Table 1988 All attempts towards explaining hybrid vigour stem from one fundamental fact, the association of vigour with the heterozygous state. Many geneticists have suggested in one form or another that heterozygosity per se is necessary for heterosis. Suppose there are alleles  $a_1$  and  $a_2$  for a single locus. The heterozygous combination  $a_1a_2$  according to a theory based on interaction of alleles is superior to either of the possible homozygotes  $a_1a_1$  or  $a_2a_2$ . It is evident that this is a sort of dominance reaction. Hence, the theory is called the theory of overdominance. As per this theory, the alleles  $a_1$  and  $a_2$  generally do separate things and the sum of their different products or some reaction product between them is superior in respect of vigour to the simple product formed by either allele in the homozygous state.

According to many geneticists, heterosis does not need overdominance. They explain it in terms of ordinary dominance of genes relatively favourable for vigour and the corresponding recessiveness of genes not favourable to vigour. tions of use any good to low off sinds

Induced Mutations Rapid progress in cytology and genetics has enabled plant breeders to devise newer methods. One of the important methods which has been employed recently concerns induced mutations. A drawback of this method is that the induced mutants usually have reduced viability which is because of the deleterious changes in the chromosome structure. In addition, there are finer changes representing true gene mutations which are useless from the practical point of view. However, there is still a remaining group of individual mutations which though extremely small is not harmful. On the contrary, it exhibits normal or greater viability. The new genes or alleles induced by irradiation or chemical mutagens are essentially similar to those occurring spontaneously. The rate of the latter kind of mutations is, in fact, accelerated by a mutagen.

The work on induced mutations has clearly indicated that mutation breeding has a positive value in plant breeding. In barley, it has been possible to induce very marked and desirable changes in respect of straw stiffness and earliness. Some of the mutants are high yielding. In India, mutational breeding has now become a favourable tool in the hands of breeders. Favourable mutants have been obtained in groundnut, rice, wheat and some other crops. They show increased yield and improvement in the quality of seed.

### **Induced Polyploidy**

Induced polyploidy has become an important tool in plant breeding. Although the majority of induced polyploids are not valuable, there still remains a group of material which exhibits favourable characters after induction of polyploidy. In such cases, this material assumes importance from the practical point of plant breeding. It is, however, necessary in most cases to improve the material of primary polyploids by recombination and selection.

Among the polyploids, both the auto- and alloploids may exhibit practical value. Diploid allogamous species whose vegetative parts are of economic importance are found to be favourable starting material for inducing autopolyploidy. Breeders have succeeded in inducing desirable polyploids in red clover and other crops. In turnip, Levan has secured good yield values in case of tetraploids. We have already seen how Muntzing made a successful cross of rye and wheat and obtained an amphidiploid rye-wheat called *Triticale*.

The above account indicates that plant breeding based on genetic principles is now of utmost importance to the economy of any country as it would solve the food problem. It should however be supplemented with proper manuring and other agricultural practices.

## Breeding for Disease Resistance

Plant breeders are greatly interested in breeding for disease resistance. In fact, many breeding programmes aim at immediate addition or recombination of disease resistance with other important agronomic characters.

We have already seen how the knowledge of genetics is applied to the improvement of plants, and how selection, hybridisation, mutation and polyploidy are used in this regard. Besides these, plant introduction is also an important method. The crops that are cultivated today originated in different parts of the world. For example, maize originally came from America and mango from India. However, they are cultivated in other parts of the world besides America and India respectively, because they were introduced in other countries having the required climate and soil. There are certain species which are disease resistant. Their introduction is advantageous.

As regards breeding for disease resistance, the very methods that are used for plant breeding (introduction, selection, hybridisation, mutation and polyploidy) used for breeding for disease resistance as well. In addition, budding and grafting are also employed.

Plant Introduction and Section And the Production of the Productio The character of disease resistance is found in different crop plants in large or less proportions all over the world. Hence, the simplest remedy is the introduction of a disease-resistant variety. It may be advantageous to cultivate a disease-resistant variety in the region where a particular plant disease occurs. However, plant introduction has its own limitations. Many a times, it is seen that a disease-resistant variety is unable to acclimatise itself to the climatic conditions of the new region. A variety resistant to a certain disease may be susceptible to the state of the s another disease. covers to the contract of mage of the particular contract of the particular and the contract of the contract o

Selection To House and the Selection of This is used in the case of introduction of a foreign variety and local varieties of a crop plant. In the beginning, plants are grown in the field and disease resistant plants are selected. The selected plants are then grown along with the susceptible plants in fields or glasshouses. They are planted en masse or in progeny rows depending on how selection is to be made of the desirous plants. The selected plants are then infected with the pathogen. Conditions favourable for the growth of the pathogen are created so that the disease can spread everywhere. The disease-resistant plants are then tested. Only those plants exhibiting disease resistance are selected. They are tested for disease resistance for several years by cultivating them in different seasons. If they are affected by the disease, they are rejected. If they remain resistant in all seasons, they are selected and cultivated in different regions under different climatic conditions. In this way, ultimately those which remain resistant through all trials are selected as disease-resistant plants. Many a times, it is seen that such plants besides being disease resistant, possess such characters which are not economically viable.

This is the best method of producing a desirable variety by crossing two different varieties. The variety under cultivation which is good except for its susceptibility for the disease, is hybridized with a disease-resistant plant with a view to introduce the resistant character in it. If the disease-resistant parent is unable to grow well under a particular climate or does not prove useful, then the method of backcrossing is used.

In the case of crop plants which do not produce sexually, hybridisation is not possible. In such a case, the graft of the disease-resistant plant is made on the susceptible plant. In some fruit plants that propagate vegetatively, the graft method is used in transferring disease resistance.

#### Mutation Breeding

This can be tried in the case of commercial crops or a susceptible variety. Although a resistant variety possesses a gene for resistance, sometimes there is a possibility of interference between hybridisation and desirable characteristics and hence it cannot be used for disease resistance. Mutation may lead to production of resistant genes and thus there may be a possibility of obtaining a resistant variety.

It will be seen from the above account that new varieties are produced by adopting (i) introduction, (ii) selection, (iii) hybridisation, (iv) mutation, and (v) polyploidy which are the principal methods of plant breeding.

## Hybridisation of Self-pollinated Crops

Among the predominantly self-pollinated crops of major importance, wheat, oat, rice, cotton, barley, tobacco, potato, pea, bean, tomato and soyabean are included. Hayes, Immer and Smith have classified the following methods to be used in improving such crop plants by hybridisation: The is used in the case of introduction of a foreign variety and local limit

- 1. Pedigree method generated has bloth and the mean our atomic administration of the business.
- 2. Bulk method and half sediments all diffe and a many many many bear and bear a said 3. Back-cross method
- 4. Multiple crosses and the horizon well the crosses of the ender of t

In this method, selection is practised in each generation from  $F_1$  onwards. The reproductive behaviour of the selected plants is then observed.

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Bulk Method and some self mass on a sense of the sense of Mass selection is made in this method. Plants selected from the F2 generation are grown in bulk and a single F<sub>3</sub> population is raised from them. Selection of the best plants is made from the  $F_3$  plants and they are again used in bulk for the  $F_4$  generation. In this way, the bulk method is followed for some years and plants breeding true to type are searched.

## Back-cross Method

The parent whose characters are to be transferred to the other parent is crossed with the  $F_1$ progeny. Hence this method is called the back-cross method. It is practised in the case of breeding for disease resistance, drought resistance and hardiness.

### Multiple Crosses

the gard of eren plants which he had produce a male hybride of When more than two parental lines having different origins are crossed, it becomes a multiple cross. It is possible to bring genes together from several parents belonging to different varieties by this method of compound-crossing. First, pairs of pure parental lines are formed and members of each pair are crossed with each other, e.g.  $A \times B$ ,  $C \times D$ ,  $E \times F$ ,  $G \times H$ , etc. The  $F_1$  progenies of different crosses are in turn crossed to give combinations:  $(A \times B) \times (C \times D)$ 

and  $(E \times F) \times (G \times H)$ . These are combined into  $(A \times B) \times (C \times D) \times (E \times F) \times (G \times H)$ . It should be noted that segregation already starts when the Fis or single crosses are combined. Hence, a very large number of crosses would be necessary in the ultimate combination for introducing all the possible gene combinations. From the above multiple cross, ultimately F2 Generation is obtained. Subsequent breeding is by pedigree or bulk method.

#### Hybridisation of Cross-pollinated Crops

Maize, rye, fodder grasses and pumpkin are some of the cross-pollinated crop plants. The best hybridisation technique in their case is the inbred line method which involves inbreeding and production of relatively homozygous lines through selection.

Suitable plants are selected from the open-pollinated plants. The progeny of these selected plants is kept under observation and only plants which exhibit desirable characters are selffertilised. In this way, self-fertilisation is practised for a number of generations. Because of inbreeding, homozygosity increases each generation. However, some undesirable characters (e.g. lethal genes, sterility, growth defects, chlorophyll deficiency) may appear due to inbreeding. After self-fertilisation for some generations, the plant becomes homozygous and breeds true. Then a few homozygous pure lines are selected on the basis of desired characters. These selected lines are then crossed. By following this method in the case of cross-pollinated crops, hybrid vigour may be displayed. The following methods are adopted in the case of selected lines

# 1. Single Cross Method (Figs. 33.1 and 33.2)

Two inbred lines are crossed  $(A \times B)$  in this method and the hybrid seed is sown for getting

## 2. Triple Cross Method (Fig. 33.3)

In this method, a combination of genes becomes effective. The single hybrid  $(A \times B)$  is used as a female parent and it is crossed with the inbred line C acting as a male parent. In this way, three inbred lines are involved in this cross. In the triple cross,  $F_1$ , which is used as a female parent, exhibits hybrid vigour from the beginning. Hence, the seed production in the hybrid is maximum and the grain is of normal size. However, the disadvantage of this method les in the difficulty of getting enough pollen from the inbred male parent. The characters exhibited by the triple cross hybrid are intermediate between the single cross and double cross hybrids. The maize hybrid Ganga 2 has been produced by this method.

# 3. Double Cross Method (Fig. 33.4)

In this method, two single cross hybrids  $(A \times B)$  and  $(C \times D)$  are crossed. So genes from these  $\sim$  varieties are combined effectively in the  $F_1$  hybrids. It is necessary to take the precaution of not including inbred lines involved in single crosses in the case of double cross parents to be used in a single cross. In maize, two single cross hybrids are alternately planted in an isolated plot of the field, and the plants used as the female parent are detasseled so that double hybridisation becomes possible. Usually the proportion of rows of female plants to those of camale plants is 4:1. It has been as often been one specious assists in but bodien exercis

The following formula is used to determine the number of double crosses possible from n inbred lines.

Number of double crosses = 
$$\frac{n(n-1)(n-2)(n-3)}{8}$$

Though double cross hybrids are more variable than single cross hybrids, their adaptivity is increased due to this variability. Their cultivation even on a small piece of land gives more yield. This is because usually double crosses which combine together two single crosses of diverse genetical make-up are more uniformly productive than those involving single crosses which combine inbreds of different parentage.

It will be seen from the foregoing account that the science of plant breeding based on genetic principles has been proved to be a very important science from the point of food supply to the whole world. For keeping humanity away from hunger, scientific plant breeding has played an important role with the assistance of fertilisers and other agricultural practices.

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# SUMMARY programmed the specific of the specifi

- The basis of the breeders' work is heredity. Though ancient man practised selection of
  plants and animals, their breeding had no conscious and scientific basis.
- 2. Even before 1900 the art of animal breeding was well advanced. Pure breeding was established as the standard method of producing speed stock. Cross-breeding and grading were practised on a large scale for the production of commercial stock. Attempts were made to use pedigrees in estimating the worth of an animal. After the rediscovery of Mendel's work, animal breeders realised the importance of genetic characters and how they behaved differently from environmental characters. An animal breeder now takes into consideration, the effect of both these characters and undertakes progeny testing. He takes into account the selection index, coefficient of inbreeding and line breeding.
- 3. There is an intimate relationship between plant breeding and genetics. The principle of genetic recombination clearly indicated to breeders the possibility of combining various characters from different strains. Johannsen's pure line experiments on selection proved to be instructive to plant breeders. The discovery of hybrid vigour or heterosis in maize led to the extensive cultivation of hybrid corn in USA. Rapid progress in cytology and genetics has enabled plant breeders to devise new methods. Induction of mutations and polyploidy are also now used in many breeding programmes besides hybridisation and selection.
- 4. Many breeding regrammes aim at immediate addition or recombination of disease resistance with other important agronomic characters. Method like introduction, selection, hybridisation, mutation, polyploidy, budding and grafting are employed in regard to breeding for disease resistance.
- In case of hybridisation of self-pollinated crops, pedigree method, bulk method, back cross method and multiple crosses are used, while in regard to hybridisation of cross-

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pollinated crops, single cross method, triple cross method and double cross method are adopted with reference to selective lines.

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To define life is very difficult because it is dynamic. It represents a continuously changing process of extreme complexity which is indicated in the organisation of living systems as compared to non-living ones.

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All living and non-living matter consists of basic units of atom which in their turn are made up of fundamental particles, protons, neutrons and electrons. A molecule comprises atoms. All non-living matter is made up of either of groups of one type of molecular substances or of several types of molecules, i.e. mixtures. Living organisms are, in fact, mixtures of very large and complex molecules which act in a coordinated manner. The largest and most complex molecules are proteins. They approach the smallest of living organisms, viruses, in size and chemical activity. We know that above the level of viruses, a living organism consists of a cell or cells which are basic units of a living organism. A cell is made up of several different types of complex molecules. So we see that every living matter consists of atoms and molecules.

All living organisms are made up of a combination of non-living substances, water, carbohydrate, lipids, proteins, nucleic acids, etc. They have the ability to break down chemical compounds so as to obtain energy for various activities.

Let us now consider the question of essential features of life. According to Muller (1929), life depends on self-reproduction and it has the unique ability to multiply variants. In other words, life must have the ability to self-copying and mutation with the mutant types able to multiply themselves. This does not mean that the earliest forms of life were able to copy themselves precisely. It is likely that in the beatining there might have been many more mistakes in the copying process. In this sense, it is conceivable that mutations might have occurred earlier in the history of life than self-reproduction. Natural selection would have selected those with the greatest ratio of correct to wrong copies. In other words, it would cause lowering of the mutation rate. So the gene through its ability of self-reproduction and to mutate must have served as the primary basis of life. Natural selection can become effective to a certain extent only when these two properties are present.

Several biologists at different times put forward their views in the form of theories on the origin of life. These are summarised below.

## 1. Theory of Eternity of the Present Conditions

This theory emphasises the unchangeability of the universe. That is, it puts forth the theory that neither was there a beginning nor will there be an end to the universe. So the

life forms existing several million years ago, have remained unchanged till the present day. They would continue in the same way throughout. This theory has been rejected.

#### 2. Theory of Special Creation

The greatest advocate of this theory was Father Suarez (1548-1613), a Spanish priest. According to this theory, God created all animals and plants. People believed in this theory till the middle of the nineteenth century.

### 3. Theory of Catastrophism

Cuvier (1769-1832), a palaeontologist, was the first to put forward this theory. He conceived the idea that the earth has been subjected at different intervals to sudden calamities or catastrophes (e.g. disappearance of the earth's crust followed by the invasion of the sea and upheaval of the mountain chains). He thought that these revolutions were local and caused the death of old fauna. Their extinction led to the creation of new fauna which took a long time, covering millions of years, due o the changed conditions of the environment. d'Orfingy (1802-1857) had stated that these calamities were not local but so extensive in the world that during each catastrophe, entire flora and fauna of the universe were destroyec and repopulation was effected only through recreation.

#### 4. Theory of Organic Evolution

According to this theory, the earth has been evolved and not created. It has arisen slowly from a small beginning and increased through the activities of elementary forces contained in itself. So life if considered to have rather grown than come into existence suddenly. Empedocles, a Greek philosopher, was the first to conceive the idea of evolution.

## 5. Respiratory Systems and Photosynthesis

It is believed that since the most primitive organisms lived in an environment abundant in most of the complex substances which organisms might require, they must have had a metabolism which was mostly of catabolic nature. In other words, the mechanism was able to break down complex substances so as to release the energy stored in them. However, when the supply of compounds with high energy became reduced in the environment, the necessity of evolving a new kind of metabolism became evident. There was, therefore, development of anabolic metabolism, and the simplest organisms like bacteria which were evolving explored possible sources of energy. Thus several types of respiratory reactions and correlated with nutritive systems exploited by bacteria, came into existence. The nutritive systems are heterotrophic and autotrophic. The heterotrophic bacteria are generally parasitic. They obtain energy by oxidation of carbohydrates or other organic compounds of the host. The saprophytic bacteria absorb dissolved organic matter from their environment. There are also autotrophic bacteria. They obtain energy from chemical reactions in which simple inorganic compounds are involved, e.g. sulphur and iron bacteria. There is, however, a The nutritive systems of both the autotrophic and heterotrophic bacteria are immensely much lower yield of energy.

inferior to photosynthesis which is the autotrophic mechanism of green plants. The evolution of photosynthetic mechanism may be the real basis for the evolution of all green plants and animals. This mechanism enables green plants to tap the abundant radiant energy from the sun. E. Theory of Special Creatless

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6. Chemical Evolution of Life and has commended boundary back and of guidance A Oparin has dealt with the origin of life in his book first published in 1924 (cf. Oparin, 1957). According to him there might have been a biogenic formation of the simplest organic substances, i.e. hycrocarbons. In his opinion, molecules of organic compounds combine to form colloid masses. These masses compete with each other to produce substances. The internal constitution of colloid masses being more adaptive, they are able to catch new molecules more rapidly than other colloid masses. Ultimately, their kind becomes dominant. At the same time, size also assumes importance. The growing colloid particle becomes unstabilised and breaks into smaller particles. Each such particle grows and again divides. Such processes occur in non-living systems and they also play a role in the creation of living organisms.

#### of the source for the state of the The Early Atmosphere and Formation of Large Molecules

It is now generally agreed that the atmosphere of the primitive earth had very little free oxygen. This is significant for the present atmosphere. Ozone is the main agent which filters out ultra-violet rays. Earlier, because of the scarcity of free oxygen, ultra-violet rays penetrated to the surface of the earth and this acted as agents for the supply of activating energy required for various chemical reactions.

It is supposed that immediately after photosynthesis began or probably due to the geological changes, the atmosphere got filled with oxygen with the result that ultra-violet rays started getting filtered out by the ozone in the upper atmosphere.

Experiments carried out in the laboratory indicate that in the presence of ultraviolet rays, ionising radiation (e.g. cosmic rays or electric discharge as from lightning), the materials that are considered to have been present then, such as H, NH2, CH4, H2O, produce amino acids, nucleotides and sources of energy like ATP. So it seems as Haldane has proposed, that mechanisms were present for forming organic compounds.

There also existed mechanisms which favoured aggregation of small organic molecules into large polymers. One of the simple probabilities in this connection is polymerisation caused by ultra-violet rays which also effect dissociation of large molecules.

Recent researches on DNA indicate that relatively simple substances can possess properties of self-copying and mutation. Thus it is probable that as soon as molecules were formed, there were considerable chances of self-copying and mutation. Subsequently, there was increase in the enzymatic and metabolic activities due to mutations and the operation The sage ophytic on term absorb dissolved at along market from their one

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This might have occurred naturally. In 1945, Horowitz suggested a specific scheme to account for its occurrence. According to him, there is present a series of chemical

compounds in the environment whose chemical properties are such that A is potentially able to be converted into B, B into C, C into D and D into E which is the material required by the primitive organism as food. When the supply of E is exhausted, there will be a strong selective advantage for any mutation that is able to convert D into E. This will supplant the others. The supply of D will then soon be exhausted. Hence, a mutant which converts C into D (while still converting D into E) will be selected. In this way, the entire chemical process  $A \rightarrow B \rightarrow C \rightarrow D \rightarrow E$  is acquired in the reverse way.

#### What is Evolution?

A study of the theory of evolution is essential for a student of biology just as a study of the atomic theory is essential for a student of chemistry or physics. Evolution pervades the entire drama of life.

The word 'evolution' refers to the gradual development of something. So we may speak of the evolution of earth, the evolution of man, the evolution of political principles, the evolution of plants, and so on and so forth. When the evolution is with reference to plants or animals or both, it is referred to as organic evolution. On the other hand, inorganic evolution is concerned with the evolution of relative fields of physics and chemistry. Broadly, evolution can be said to be the development of an entity in the course of time through a gradual sequence of changes from simple to a more complex state. Darwin defined it as "descent with modification". According to Zimmermann (1953), evolution is the transformation of the form and mode of existence of an organism in such a manner that the descendants differ from their ancestors.

According to the theory of evolution, all forms of life living and extinct have originated from pre-existing ones by a process of gradual change. So the process of organisation of different kinds of plants and animals by descent from other kinds takes place through the interaction of natural forces in the evolution. One definition of evolution is that it is a change in the genetic constitution of a population. However, we should consider only the evolutionary change which exhibits the hereditary characteristics of the population as a whole.

#### **Evidence of Evolution**

There is no doubt that species have evolved through the process of evolution. Darwin in his monumental work, Origin of Species proved convincingly that species have been evolved. In support, he cited various evidences from different fields. These include evidences from (i) anatomy, (ii) taxonomy, (iii) serology, (iv) embryology, (v) palaeontology, (vi) geographical distribution, (vii) genetics, (viii) vestigial organs, and (ix) phylogeny and biochemistry. We shall deal with some of them.

The ancestry of the horse can be traced back to a small animal that possessed four toes on its front legs (instead of the single toe with a large hoof of the present dry horse). This for mode for example year an ince triplet a fairness distantly related to crayish but

creature existed in the eocine period (Fig. 34.1). As we pass to higher layers of stratified rocks, we note that the horse gradually acquired its modern form. There was a change from four toes to three toes by eliminating the second and fourth toes and reduction of the second and fourth hand bones to splints. Simultaneously, there occurred another change. Its middle hand bone between the wrist and the middle finger became very long and an extra joint was formed in its legs. Besides its teeth gradually changed from the low-crowned chewing type to the high-crowned grinding type present in the modern-day horse.

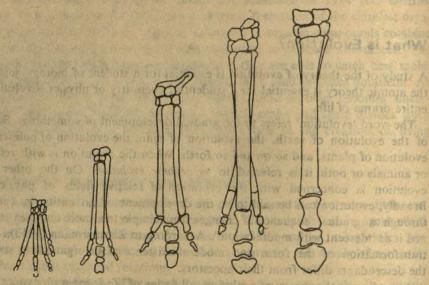


Fig. 34.1 Formation of front leg of horse.

We now know that the stratified rocks were laid down from below up. They were originally formed as sediment which was washed from higher to lower parts of the land. The lower layers were deposited first and the higher layers later. The sequence in the case of horse fossils is found to start below and then rises upward in the stratified rocks. It (sequence) corresponds to that of the stratified rocks in time. If the fossils are arranged in this time sequence, it is clear that the sequence is evolutionary.

There has been an exhaustive study of the palaeontology of horse. The horse series is based on the study of innumerable fossil specimens. Frequently, it is seen that one member of the series is so much similar to the one below or above it in the series that it is difficult to detect any difference between them. The change in the horse as indicated by the series of fossils is very gradual. The fossil material is in plenty which points out that large herds of horses wandered over North America in prehistoric times. They died under such circumstances which favoured their fossilisation.

Like the horse series, there are other series of animals like the elephant, camel and titanotherus (now extinct) which also convincingly indicate evolution of these animals. The same is the case of many invertebrate forms in which there is abundant material available for study, for example, very ancient trilobites (animals distantly related to crayfish but antidating it by millions of years), snails and ammonites (extinct animals having snail-like shell but coiled after the manner of watch spring).

#### **Evidence from Embryology**

Some data on paths of evolution are available from embryology. Every living being has a tendency to multiply. The multiplication takes place either by sexual or asexual reproduction or by both. Several plants or animals belonging to different groups show remarkable similarity at the embryonic stages, thus making it difficult to recognise them till later stages. This similarity at the embryonal stage is indicative of their origin from the same ancestor.

It has been observed that seedlings sometimes resemble their ancestors. For example, the Acacia tree possesses highly compound leaves, but its seedlings have simple leaves like its ancestors. An adult Cactus plant does not bear typical leaves at all. These are represented by spines. But its seedlings possess leaves.

There is sometimes a remarkable resemblance in the case of some organs of animals or plants to certain forms from which they might have originated (Fig. 34.2). In bryophytes and petridophytes, spores on germination form filamental structures which resemble filamentous algae. This fact suggests that the ancestors of these plants were algae. With the help of such studies, it is possible to trace the evolutionary history of a species. This study indicates that ontogeny recapitulates phylogeny. In other words, the development of an individual repeats the ancestry of the race.

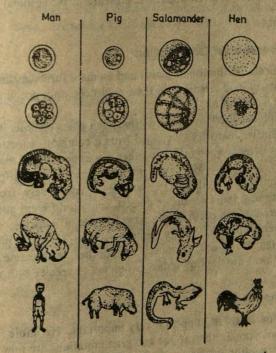


Fig. 34.2 Comparison of stages of development of embryo in man, pig, salamander and hen.

#### **Anatomical Evidences**

We shall now consider some of the direct evidences derived from the study of comparative anatomy. If we superficially examine the arm of a man and the forelimb of a horse, they will look very different. But on closer examination, it will be seen that there is a basic similarity below the surface. We have upper and lower arm bones, wrist bones, hand bones and finger bones. The horse also has corresponding bones in its foreleg. But one may ask why should there be this correspondence since these two organs have altogether different uses. There cannot be any reason to be advanced on functional ground. Why should they be basically similar? If man and horse had evolved from a common ancestor which possessed the basic plan of the limb structure common to both, then it becomes apparent about the similarity in the basic structure of the forelimb of man and horse. They might have derived this structure from their common ancestor. It can be then assumed that the differences in their limb structure might be because of divergence in the direction of their evolution.

A study of comparative anatomy indicates that some animals possess mere vestiges of organs which are found to be well developed in other animals. For example, the whale has no hind limbs which are outwardly visible, but there are vestiges of hind limbs below the skin. They are functionless as far as we know. So the question is why they should be present. The answer is they represent remnants of structures which were once functional in the ancestry of whale. This means that the presence of a vestigial organ is indicative of the previous presence in a line of descent of a corresponding functional organ. This is the only rational explanation that can be given about the presence of the vestigial hind limbs of the whale.

#### **Evidences from Geographical Distribution**

The study of geographical distribution of plants and animals furnishes the most convincing proof of evolution. Darwin paid attention to these evidences and accounted the possibility of the origin of species by evolution.

The study of geographical distribution shows that each place exhibits its typical flora and fauna. This feature indicates that they originated from a common ancestor existing in the region. They could not exceed in migrating to other places because of various barriers such as mountains, deserts and seas. Moreover, there are reports that different regions which have the same environmental conditions but are separated widely from each other by barriers like high mountains, large oceans or deserts, contain different species. This implies that the different species which occur in the same latitude of widely separated land masses do not owe their differences to either soil or climatic factors. There are plants which can be successfully transplanted from one continent to another in the same latitude. Thus, the evidence from geographical distribution indicates descent of species with modification.

#### **Evidences from Genetics**

As genetics deals with heredity, its support is very important. Heritable variations accomplished through chromosomal aberrations and gene mutations are now known to be basically responsible for speciation or descent with modification.

#### **Evidences from Comparative Physiology**

A strong support also comes from the field of comparative physiology in mammals. The organs of digestion in vertebrates are similar in structure and location. They also exhibit much similarity in function. The enzymes formed by them are chiefly similar. The parts of the central nervous system perform more or less the same functions. The chemical reactions that take place in respiration are basically similar in most diverse organisms. Most animals are able to oxidise uric acid readily to more soluble allantoin which is eliminated from the body along with urine. However, man is not able to do this and hence he is susceptible to gout. In this inability, he resembles tailless apes.

Blood serum tests also give a convincing evidence in favour of evolution. Animals which seem to be closely related on the basis of other grounds, have been found to show similar types of blood. The blood of a horse is similar to that of an ass, while that of a rabbit is similar to that of a hare, etc. Man's blood is similar to that of anthrapoid apes (like chimpanzec).

#### Historical Ideas of Evolution

Genetics has contributed to the study of evolution in furnishing an explanation to the manner in which evolution takes place. Let us ignore all theories which imply purposeful evolution or require some supernatural force since they cannot be tested by scientific methods. So the theories that are of fundamental importance in connection with evolution will be considered here. These are based on observations and ideas of the following four men.

#### Lamarck (1744-1829)

He propagated the idea that acquired characters and the effects of use and disuse are inherited. So according to him, an animal which had exercised a great deal and developed strong muscles would have offspring with stronger muscles. An organ not in use would atrophy and in the next generation it would be smaller. A man who had spent a great part of his time outside and thus acquired a suntan would transmit this character to his offspring.

Lamarck's theory of inheritance of acquired characters gained popularity mainly because of its success in 'explaining' adaptations of plants and animals. Progressive evolution would be expected on the basis of his theory. However, numerous laboratory experiments carried out in the past fifty or more years have proved practically without exception that acquired out in the past fifty or more years have proved practically without exception that acquired out in the past fifty or more years have proved practically without exception that acquired out in the past fifty or more years have proved practically without exception that acquired out in the past fifty or more years have proved practically without exception that acquired out in the past fifty or more years have proved practically without exception that acquired the marker's characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, there are many objections, one of them is that it is characters are not inherited. Besides, the objections, one of them is that it is characters are not inheri

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#### Darwin (1809-1882)

In the pre-Darwinian era, biologists and philosophers had some notion about evolution but they had very little proof in its favour. Hence, Darwin is called the Father of evolution. He collected an appreciable amount of evidence from all branches of biology in support of his theory of evolution and presented this in his magnum opus Origin of Species (1859) so convincingly that a large number of biologists came to accept the idea that species originated through evolution.

Darwin also developed the theory of natural selection (and independently by Wallace) for accounting the adaptive nature of evolution. In Darwin's time, no sharp distinction was made between heritable and non-heritable variations (between mutations and acquired characters). This distinction was established mostly by Johannsen on the basis of his work on pure lines. Darwin's important contribution was in regard to variations. This helped to understand the occurrence of adaptive evolution. Darwin considered variations as random or fortutious. According to him, in the ancestry of horse, variations in respect of speed did not turn up in response to the needs of the horse. There were all kinds of variations and only those which were beneficial (in this case, increased speed) survived. The study of mutations offers support to the fortuitous nature of variations. Now, we know that only mutations in the ultimate analysis represent heritable changes and adaptive evolution occurs through the process of natural selection. In other words, it comes through the survival of beneficial mutations and destruction of unfavourable ones under the competitive conditions of over-reproduction occurring in nature.

At the time of Darwin, one of the chief hurdles to the acceptance of the theory of evolution was the fact that all living beings were found to be remarkably adapted to their environments. On the basis of this fact, biologists thought that all forms of life were created by intelligent design. Darwin's theory of natural selection overcame this hurdle.

In any species of plant or animal, the tendency of the reproductive is to outrun the available supply of food. The resulting competition leads to a struggle for existence. Only a small portion of all the individuals born can survive to produce offspring. As per the theory of natural selection, the nature of the surviving portion is not determined by chance alone. Any two individuals of a species are not precisely similar and of the variations which occur, some of them enable their owners to face the existing competitive conditions comparatively in a more successful way. Hence, they will have a better chance of survival in the struggle for existence as compared with their less favoured brethren. In other words, there will be survival of the fittest and such individuals will have a better chance of leaving offspring. Darwin introduced the maniple of heredity in his theory. He assumed that the tendency of offspring was to resemble their parents more than other members of the species. Parents having a beneficial variation tend to transmit this variation to their offspring, to some in larger, in others in lesser proportion. Those possessing it in larger proportion will again have a better chance of survival and they will transmit the beneficial variation in even greater degree to some of their offspring. Thus, a competitive struggle for existence working in combination with certain principles of variation and heredity leads to a slow and continuous transformation of species through a process which Darwin called natural selection. Because of virtues of coherence and simplicity possessed by this theory of natural selection and supported by a large number of facts marshalled together by Darwin, it received recognition rapidly from a great majority of biologists.

Darwin provided a natural explanation in the form of his theory for adaptation. He showed that a supernatural being was not necessarily the only possible one. He laid a firm foundation for the science of evolution.

#### Pangenesis Theory

Darwin knew that his theory of evolution lacked the evidence of heredity. Therefore, he proposed the pangenesis theory by which he tried to explain inheritance of acquired characters. He assumed that hereditary materials were drawn from all parts of an organism and he termed them as 'pangenes'. These produced germ cells, which, in turn, gave rise to new individuals. This theory of Darwin was severely criticised. Darwin was also not able to distinguish between germinal and somatic variations.

#### Objections to Darwin's Theory

Even today, there are non-biologists who are under the impression that the natural selection theory is the modern theory of evolution. However, this is not the case. In fact, Darwin's theory has been criticised on the following grounds.

- 1. Darwin was not able to distinguish between germinal and somatic variations,
- 2. He did not know the causes of variations.
- 3. He thought that variations occur in all directions at random but at present it is assumed that they occur only in definite lines of change.
- 4. He had no knowledge about heredity.
- 5. His theory of pangenesis is not supported by any evidence in its favour.
- 6. How could slight individual variations have selection value?
- 7. Johannsen's pure line experiments indicate that natural selection is ineffective in a pure
- 8. According to Darwin only useful characters are inherited. However, certain useless and non-adaptive characters are also transmitted.
- 9. It is really difficult to explain how natural selection could make use of initial stages of adaptive structures.
- 10. It does not account for overspecialisation and degeneration.
- 11. Darwin's theory explains the survival but not the arrival of the fittest.
- 12. If nature selects suitable forms and characters, then the question that arises is why were the rest not removed from existence.

#### Weismann (1834-1919)

He was a German zoologist and a disciple of Darwin. He opposed Lamarck's theory of inheritance of acquired characters as well as Darwin's theory of pangenesis. He proposed the germ plasm theory. According to this theory, the body of a living organism consists of somatoplasm giving rise to body or somatic cells and germ plasm forming reproductive cells. The germ plasm is continuous from generation to generation, while somatoplasm is discontinuous. The latter is formed anew in each generation. Weismann expressed the opinion that germ cells are also concerned in evolutionary changes. The theory was later criticised since there was no evidence to support it. However, he must be given credit for clearly distinguishing between germ cells and somatic cells. He pointed out that effects on somatic cells do not ordinarily influence the germ cells and so they are not transmitted to the next generation. His famous experiments of cutting off tails of mice for many generations and demonstrating that descendants were still born with tails helped to discard Lamarck's theory of inheritance of acquired characters.

#### De Vries (1848-1935)

We already know that De Vries was one of the three persons who discovered Mendel's work. In addition, he is known for his mutation theory. He was the first to realise the role of mutations in evolution.

The variations considered by Darwin as a force in evolution were minute fluctuations or continuous variations. He did not think that large variations called 'sports' by him were of any significance in evolution. De Vries, on the other hand, proposed that new races and species originate discontinuously and not gradually. He regarded fluctuations as dependent on nutrition and as such did not give rise to any permanent change. For instance, cultivation and improved nutrition give rise to any minor differences in size and luxuriance of reproductiveness in plants. Selection acting on these fluctuations may be able to alter the average condition of the race. However, such changes will persist only so long as the conditions which were responsible for their formation, are maintained. No sooner selection ceases to act than the race gradually returns to the former condition. De Vries considered a permanent racial change not as a product of a fluctuating variability. According to him, it arises from a discontinuous variation or sport which he termed mutation. Mutations are sudden heritable changes. They occur in organisms in unpredictable directions. They are not caused by environmental effects or cross-breeding. De Vries suggested that mutation effected a change in the nature of germ cells while fluctuation was because of the effect of environment. The soma as well as germ plasm may be modified by environment but it will be only temporarily. He thought that permanent changes take place spontaneously out of the internal conditions and are not necessarily adaptive to nature. Majority of them get destroyed since they are not beneficial (non-adaptive). Only those mutations that are adaptive survive in nature. The role of environment is only to determine which mutation will survive. According to De Vries, only large and discrete mutations were important in evolution and small mutations did not have any major role. He thought that the new species appear all at once. Their origin from the parent species is sudden and in a single step without any series of intermediate forms. He recognised natural selection as a factor in evolution but assigned to it a limited function of eliminating forms which are purely adapted to environment.

De Vries' mutation theory is based on his experimental work on Oenothera lamarckiana. In 1886, this plant was growing wildly in a deserted potato field at Hilversum near Amsterdam in Holland. He observed that the plant exhibited remarkable variability. During the period of several years, he found more than a dozen new types. Some of them were new species, for instance, O. brevistylis possessing characteristic short style of the carpel, O. nanella characterised by its dwarfness and O. gigas being stouter and having larger leaves and flowers. Most of the mutants were stable and never reverted to the original O. lamarckiana type.

Later researches indicated that what De Vries considered as 'mutations' were in fact segregations from complex chromosomal rearrangements. Most mutations produce much smaller

effects than conceived by De Vries. However, credit must be given to him for indicating that mutations are the source of variability on which natural selection acts.

#### Theory of Orthogenesis

Evolution tends to occur in certain directions which are generally adaptive. It is determined completely by tendencies within the organism. For instance, according to this theory, the ancestors of the horse evolved longer legs because of some inner tendency on the part of their germ plasm to form longer legs. It appears superficially that the theory is supported by palaeontological evidence. However, it may be that evolution of the horse might have taken place because of mutation and natural selection. Any series of fossils which is adaptive could be generally interpreted in the same manner. The theory of orthogenesis fails to explain why evolution proceeded along the lines of adaptation. The study of present forms of life does not give any conclusive evidence in support of this theory.

#### Origin of Variation

important as well as a characteristic feature of all living organisms is heredity which enables 'like to beget like'. Heredity is a conservative force. This does not mean that the offspring could be an exact copy of their parents. Otherwise there would not have been any evolution. Heredity is associated with an opposing force, that is, variability.

When we study evolution, we have to deal with the manner of origin of hereditary variations. Lamarck was the first to make an attempt in this direction. However, his theory of the effect of use and disuse and inheritance of acquired characters stands rejected. Darwin did not consider this problem. He was of the opinion that all variations were inheritable.

A great deal of progress has been made in the field of cytology and genetics in the last 40 years. We now know that self-reproduction occasionally gives rise to a changed or imperfect copy of the parent, i.e. mutation. Heredity and variation go hand in hand. The units of heredity and mutation are the same. They are genes. Mutations produce changes in them while heredity preserves them by having their faithful self-reproduction till they are further while heredity preserves them by having their faithful self-reproduction till they are further changed by the occurrence of new mutations. Thus, mutations serve as the raw material for changed by the occurrence of new mutations, chromosomal aberrations and polyploidy.

## **Contemporary Evolution Theory**

The fundamental concepts of Darwin are now accepted by almost all biologists. They have been modified in the light of present knowledge and crystallised in the form of a contemporary evolution theory. The salient features of this theory are given here.

1. Gene mutations together with chromosomal arrangements and probably position effects occur at rates that can be predicted. However, this occurrence is by chance in unpredicted individuals. They are the source of variation.

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- 2. The pool of variation is increased through the process of meiosis and fertilisation which give an opportunity to mutants to combine in various ways.
- 3. Genes responsible for characters favouring survival of individuals tend to increase in relative frequency whereas the frequency of harmful genes decreases. In this way, natural selection comes to play its role.
- 4. The process of natural selection leads to a gradual change in the species and succeeds in being better adapted to its environment with the passage of time say over millions of generations, and evolution has been achieved.

#### **Factors Affecting Evolution**

- 1. Rate of mutation: Since evolution is ultimately dependent on mutations, a certain amount of mutation is essential for the occurrence of evolution. However, it seems that the rate of mutation is not a limiting factor in evolution. Given any mutations at all, the direction and spread of evolution is mostly governed by population structure and selection.
- 2. Selection: The moulding factor in the development of species is really natural selection which decides which mutants will survive and increase and which ones will be eliminated from the population. Thus, it determines the direction of evolution of a population.
- 3. Chance: Random fluctuations in genes are specially significant in small populations. It may be that certain genes being particularly neutral in selective advantage may become stabilised or lost in the population only due to chance.
- 4. Population structure and migration: The main effect of the size of population is the part that it permits chance to play in evolution. When the population is smaller, the effect of chance is greater. The subsequent course of evolution is affected in the case of randomly mating large population or the one broken up into subunits with infrequent cross migration.

The theory of evolution is now a completely mathematical theory and perhaps in biology there is no such other theory which is completely mathematical. When it is reduced to its most basic terms, evolution can be defined as the change in the relative frequency of genes. It is largely because of the efforts of Haldane, Fisher and Wright that the theory of distribution of gene frequencies has been formulated. This distribution is considered under the influence of mutation, selection, migration and chance. Because of the development of this theory, the study of evolution has become a quantitative science. It has been possible to fit numerous and diverse facts into a common theoretical framework.

#### Rates of Evolution

It is possible to determine evolutionary rates in the case of changes in bone length and size of teeth or changes from the palaeontological records. However, no method was available until recently to find out the rate of change in actual gene. This has become possible to some extent with the help of protein chemistry. For intance, the alpha chain of haemoglobin has 141 amino acids. Of these, 17 are different in man and horse. If these species diverged from a common ancestor, as is thought, about 130 million years ago, this is equivalent to evolution

at the rate of one amino acid substitution every 15 million years. As each of these changes corresponds to one mutational step, i.e. one nucleotide change, the evolution of these genes might have been at the rate of replacement of one gene every 15 million years. Suppose that man possesses about 15,000 pairs of genes. Then if the haemoglobin genes are typical, the rate of evolution will be one gene substitution every 1000 years. Of course, more knowledge is still necessary to find out whether the haemoglobin is typical or abnormal.

# SUMMARY

- 1. Living organisms are mixtures of very large and complex molecules which act in a coordinated manner. Proteins are the largest and most complex molecules. They approach the smallest living organisms, viruses, in size and chemical activity. Life must have the ability of self-copying and mutation, with the mutant types able to multiply themselves. The gene, through its ability to self-produce and mutate, must have served as the primary basis of life.
- 2. There are various theories on the origin of life, e.g. the theory of eternity of the present conditions, the theory of special creation, the theory of catastrophism, the theory of organic evolution, autotroph versus heterotroph theory and the evolution of autotrophy from heterotrophy.
- 3. According to Oparin, natural selection has an important role in organic evolution. The processes involving formation of colloid masses and their kind becoming dominant and breaking into smaller particles occur in non-living systems. Oparin thinks that such processes also play a role in the creation of living organisms.
- 4. The theory of evolution states that all forms of life have originated from pre-existing ones by a process of gradual change. Darwin proved convincingly that species have been evolved. In support, he cited evidence from different fields. He also developed the theory of natural selection (and independently by Wallace) for accounting for the adaptive nature of evolution.
- 5. To account for the causes of evolution, Lamarck proposed the theory of inheritance of acquired characters. It stands rejected today.
- 6. De Vries was the first to realise the role of mutations in evolution. He formulated the mutation theory. He proposed that new races and species originate discontinuously and not gradually.
- 7. According to the contemporary evolution theory, for evolution to occur, there must be variation. Mutation gives rise to variation whose pool is increased through the process of meiosis and fertilisation which give opportunities to mutants to combine in various ways. Natural selection acts on such variations. It then leads to a gradual various ways. Natural selection acts on such variations. It then leads to a gradual various ways and succeeds in being better adapted to its environment with the change in the species and succeeds in being better adapted to its environment with the passage of time. Rate of mutation, selection, chance, and population structure and migration are the factors affecting evolution.

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# 35. Species Formation

Species are regarded as fundamental biological units. We now know a lot about the nature of species and the mechanisms responsible for the origin of species from races. This is mainly because of the significant contributions made by genetics in recent years.

# Reproductive Isolation and Speciation

As a rule, races of sexually reproductive species are geographically isolated. In other words. they are separated in different regions. Geographical isolation enables races to maintain their distinctness as populations and to accumulate more genetic variations. Genetic divergence may ultimately change races into reproductively isolated populations. In other words, new species are formed. Because of reproductive isolation, related species frequently occur side by side in the same region. Thus, development of reproductive isolating mechanisms between divergent populations leads to transformation of races into species. A reproductive isolating mechanism consists of a generally controlled agency which limits or prevents interbreeding of natural populations.

# Partial or Complete Isolation

It should be borne in mine that any of the reproductive isolating mechanisms may be either complete or partial. Hence, sexual isolation may result in a slight preference in the case of mating with representatives of the same species. Sometimes, it may be so strong that mating of females of one species with males of another species does not occur at all or very rarely as an exception. If mating takes place, the resulting hybrid may be constitutionally weak or inviable or may seldom survive. Besides, the exchange of genes between related species is generally prevented not only by a single isolating mechanism but also by the coordination of several mechanisms which strengthen each other.

The joint action of several reproductive-isolating mechanisms may sometimes result in suppressing hybridisation completely or sometimes there may be only partial suppression. So there will be no gene exchange between species populations. Interspecific hybrids are usually found in nature rather exceptionally. However, hybrids of Bufo americanus and B. fowleri occur somewhat regularly whenever two species occupy the same region.

#### Introgressive Hybridisation

When there is occasional hybridisation of partially isolated species, genetically interested results may be obtained. There may be naturally occurring highly variable populations. Particularly, in case of plants, such populations consisting of segregating progenies of species hybrids may inhabit places at geographic boundaries which separate regions of related species in regard to distribution. For example, *Tradescantia canaliculata* and *T. subaspera* are ecologically isolated species. They intercross in such places where their habitats merge into each other.

## Morphological and Genetical Differences between Species

Races of species and different species of a genus possess different gene complexes. So they are best fitted in respect of survival and reproduction in different habitats. Generally, the genetic differences appear in respect of such characters as colour, size and characteristic of different parts of body. The external appearance of these genotypic differences helps in identifying every individual as belonging to a particular race of a species.

# Differences between Species in Respect of Number and Form of Circumosomes

Species of a genus or a family possess simple or different numbers of chromosomes. A polyphola series is the best example of a special case of variation in chromosome number. The chromosome numbers of such a series represent a multiple of some basic number. For instance, species of wheat and related grasses possess 7, 14 and 21 chromosomes. We have already seen that polyploid series are common in several crop plants. They are, however, rare in animals. We also know that translocations can also cause changes in chromosome numbers.

Inversions produce changes in the arrangement of genes. These very seldom lead to v.able alterations of the shape of chromosomes. Such alterations are possible in the case of some pericentric inversions when there is a shift in the kinetochore position in regard to the chromosome ends.

# Allopatric and Sympatric Speciation

Speciation includes either allopatric or sympatric isolation. Allopatric speciation is the primary method of the origin of species. When two populations live in different areas and are thus separated from each other, they are prevented from interbreeding. In such a case, allopatric speciation occurs. On the other hand, in the case of sympatric speciation, two populations live in the same area. They are, however, reproductively isolated from each other. So the origin of sympatric species is by the development of sudden and direct

reproductive isolation between segments of the same population. Polyploidy, hybridisation, self-fertilisation and apomixis are the principal methods which help in sympatric speciation very common in higher plants. In the case of development of allopatric species, the separation continues for a very long period and there is interaction of variation, selection and drift in respect of each population independently. Thus, the divergence is complete.

#### **Phylectic Evolution**

In this kind of evolution, one species is transformed into another with a change in its average characters so as to achieve better adaptation to the prevailing environment which is also changing (Simpson, 1953). There is no increase in the number of species. There is only a change of one species into another with the passage of time. In this case, there is no development of reproductively isolated populations.

The foregoing account indicates that the process of formation of species consists of slow divergence of isolated populations over a number of generations. If there were no isolation, the accumulated differences would be broken down by hybridisation. Geographical isolation and later cross-sterility prevent this and so newly originated species are able to follow separate evolutionary courses. Perhaps, the formation of a new species in a single generation through polyploidy is an exception to the slow process of speciation. When chromosome doubling occurs, plants fertile among themselves but not with their diploid parents are formed. In this way, they become immediately isolated.

#### Genetic Drift

Drosophila, the carrier of different chromosomal types possesses different adaptive values. The chromosomal races occurring in fruitflies are probably representative of adaptive responses of the species to the differences in environment in relation to their habitats in different regions of the geographic distribution area. In the case of man, predominantly dark skinned populations are found living in hot regions where there is intense sunshine, whereas light skinned, fair-haired and blue-eyed populations occur in cold regions with extensive cloudiness. So it appears that there is correlation between human pigmentation and climate (although there is still no conclusive proof). On this basis, it can be assumed that dark pigmentation possesses (or at least when human races were originating) a survival value in sunny and hot regions while light pigmentation in cloudy and cold regions. Then the question about racial variations in adaptively neutral character arises.

The frequency of alleles in the gene pool of a population remains constant in time. However, it is changed by factors like mutation, selection or differential migration. Absolute consistancy can also be possible in the case of ideal infinitely large populations. Actually, however, populations are composed of finite numbers of individuals. This means that the frequency of alleles say A and a in the gene pool will remain constant from generation to generation in the case of a large population whereas it will be much variable in a small population.

Genetic drift is caused by variations in gene frequencies which arise because of sampling

errors in finite populations. Wright has indicated that if several small populations have at some time similar gene frequencies, they may become different in consequence of genetic drift with time. So also in the case of any population, if its size is small, gene frequencies may be altered with time. Genetic drift may then cause differences in the frequencies of adaptively neutral genes among small populations.

# SUMMARY TO ADDITION OF THE PROPERTY OF THE PRO

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1. Species are regarded as fundamental biological units. Geographical isolation enables races to maintain their distinctness as populations and to accumulate more genetic variations. Genetic divergence may ultimately change races into reproductively isolated populations. Thus, new species are formed.

2. Any of the reproductive isolating mechanisms may be either complete or partial. The joint action reveals that reproductive isolating mechanisms may sometimes result in

suppressing hybridisation completely or partially.

3. There may be occasional hybridisation of partially isolated species. This is known as

introgressive hybridisation.

4. There are morphological and genetic differences between species. Races of species and different species of a genus possess different gene complexes. So they are best fitted in respect of survival and reproduction in different habitats.

Differences exist between species in respect of number and form of chromosomes. A
polyploid is the best example of a special case of variation in chromosome number.

6. Speciation includes either allopatric or sympatric isolation. The former is the primary method of origin of species. In the latter case, although two populations live in the same area, they are repre fuctively isolated.

7. As regards phylectics evolution, one species is transformed into another with a change in the average characters so as to achieve better adaptation to the prevailing environ-

ment, which is also changing.

8. Genetic drift is caused by variations in gene frequencies which arise due to sampling errors in finite populations. It may cause differences in the frequencies of adaptively neutral genes among small populations.

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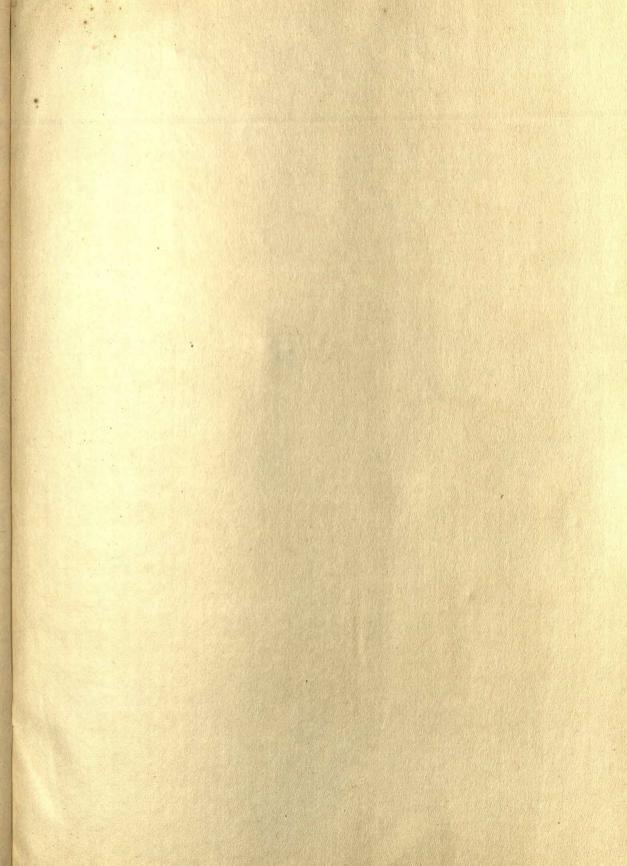
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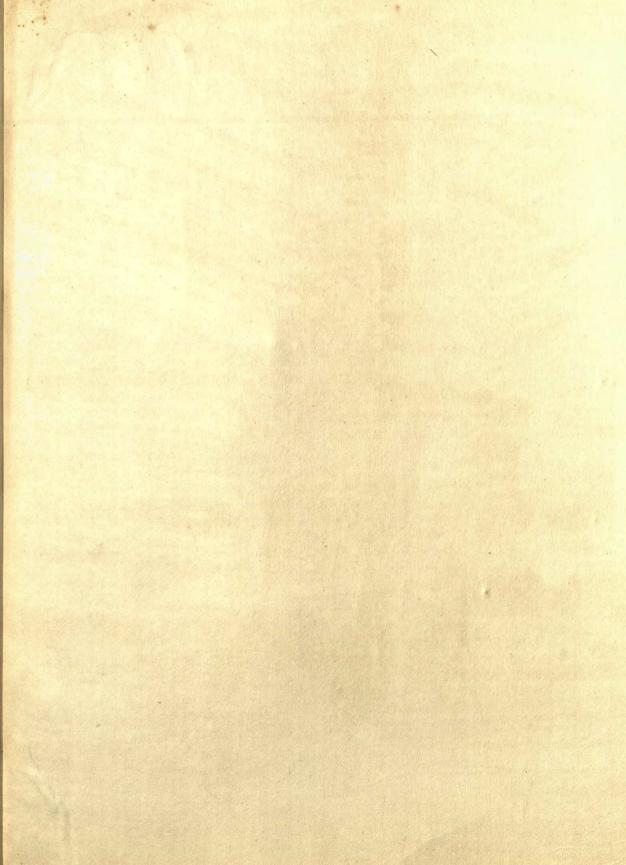
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#### V R DNYANSAGAR

retired as Professor and Head, Botany Department, Nagpur University. At present, he is President of the Society of Cytologists and Geneticists, India. An eminent scholar, he received his MSc and PhD degrees from Nagpur University. Later, he also acquired a PhD from Wisconsin University USA.

Dr Dnyansagar has a teaching experience of over 32 years. He is a Fellow of the Indian Botanical Society, Founder-Fellow of the Maharashtra Academy of Sciences and Fellow of the Indian Palynological Society. He was the President, Botany Section, Indian Science Congress, Varanasi, 1981 session and also the host President of the Bombay Biological Association. He was the Editor and Chairman of the Editorial Board of the Journal of Biological Science for several years.

He has published over 100 research papers, written one book and edited two. He has been the principal investigator of several research schemes and has guided 31 PhD students. He was awarded the V Puri Gold Medal by the Indian Botanical Society in 1980 for distinguished research work.

Tata McGraw-Hill Publishing Company Limited 4/12 Asaf Ali Road, New Delhi 110 002